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Purification and Characterization of Gut Alkaline Proteases from some Lepidopteran Larvae

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Abstract: Alkaline proteases of lepidopteran insects viz., castor semilooper *Achaea janata* Linn., American bollworm *Helicoverpa armigera* Hübner, diamondback moth *Plutella xylostella* Linn., Bihar hairy caterpillar *Spilosoma obliqua* (Walker) and tobacco caterpillar *Spodoptera litura* Fabr. were partially purified and characterized for their kinetic parameters using different substrates and inhibitors. Gel chromatography of the crude midgut extract increased the total specific activity depending upon the insect species. The protease activity of the purified midgut extract required an optimum temperature of 40°C, pH of 9.5 and incubation time of 10 min with casein as a substrate. The K_m values of proteases of different insects were also determined. The trypsin-like nature of the enzyme was confirmed by using synthetic substrates and standard protease inhibitors. The molecular weights (Kd) of the purified midgut extracts were 30 for *H. armigera*, 29 for *A. janata* and *S. litura*., whereas *P. xylostella* and *S. obliqua* extracts showed the presence of two major bands of proteins having a molecular weights of 56 and 29 kd; and 40 and 29 kd respectively.

Keywords: *Helicoverpa armigera, Achaea janata, Spodoptera litura, Plutella xylostella, Spilosoma obliqua, trypsin, protease*

INTRODUCTION

Insect proteases are important enzymes, existing freely in the lumen or bound to the microvillar membrane, that proteolyze different kinds of proteins in a variety of insect species (Applebaum, 1985; Terra, 1988). These proteases degrade proteins in highly alkaline conditions of midgut which is characteristic of most phytophagous lepidopteran insects. These are being extensively investigated in recent years in view of their importance as target sites for protease inhibitors. These protease inhibitors are found to inhibit insect growth and development and the possibility of their use as insect control agents is considered high. Addition of protease inhibitors in the diet caused growth inhibition in lepidopteran larvae (Shukle and Murdock, 1983; Broadway and

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Duffey, 1986; Broadway and Duffey, 1988; Broadway, 1989; Larocque and Houseman, 1990). Besides these could also be used in combination with other insecticides. MacIntosh et al (1990) reported synergistic effects of *Bacillus thuringiensis* endotoxin and protease inhibitors. Alternatively, proteinaceous protease inhibitors being products of single gene are thought to be ideal for development of transgenic crops (Laskowski and Kato, 1980; Ryan, 1989). The development of resistance in transgenic tobacco expressing protease inhibitor against the tobacco budworm and other lepidopterans has already been reported (Hilder et al, 1987; Johnson et al, 1989; McManus et al, 1994). To continue further advances in the development of protease inhibitors as pest control agents, it is essential to know in greater detail the characteristics of proteases in the key lepidopterous insects. Studies carried out by Ahmad et al (1980) showed the presence of 3 different proteases with molecular weights ranging from 18 to 50 kd and of trypsinogenic nature in the tobacco caterpillar. Similarly Johnston et al (1991) characterized proteases of larvae of American bollworm and reported its molecular weight of 24 kd. Christeller et al (1992) extensively investigated dietary and protease inhibitor interactions with midgut proteases in 12 phytophagous lepidopterous insects and showed presence of trypsin, chymotrypsin, elastase, and carboxypeptidase A and B, and leucine aminopeptidases; the first one as predominant one. Broadway (1995) reported proteases of 6 lepidopterous insects including diamondback moth and also its inhibition by serine proteinase inhibitors from cabbage. The castor semilooper, *Achaea janata* Linn., the American bollworm *Helicoverpa armigera* Hübner, Diamondback moth *Plutella xylostella* (Linn.), Bihar hairy caterpillar *Spilosoma obliqua* (Walker) and tobacco caterpillar *Spodoptera litura* Fabr. are the important lepidopterous pests of agricultural crops in India and elsewhere. Of these, *H. armigera*, *S. litura* and *S. obliqua* are polyphagous while *A. janata* and *P. xylostella* are oligophagous pests. Except for *H. argmigera* and *S. litura*, very little information on proteases of other insects is available. The present study therefore reports some molecular characteristics and kinetic parameters of the proteases of these insects using different substrates and their interactions with inhibitors.

MATERIALS AND METHODS

Test Insects

H. armigera larvae collected from the Pigeon pea fields were reared individually in plastic tubes in the laboratory on artificial diet at 27°C and 70% r.h. according to Nagarkatti and Prakaash (1974) with minor modification. *S. litura*, *A. janata* and *S. obliqua* larvae collected from the fields of castor were maintained on fresh clean castor leaves at 27°C and 70% r.h. under 12 h light and 12 h dark regime. *P. xylostella* larvae collected from the infested cabbage fields were reared on cabbage leaves at similar conditions as the above insects. All the adult moths were fed 10% honey solution fortified with 0.6% ascorbic acid and 0.1% α-tocopherol.

Chemicals used

The various synthetic substrates such as N-a-benzoyl-l-arginine ethyl ester (BAEE), N-benzoyl-l-tyrosine ethyl ester (BTEE), N-α-p-tosyl-l-arginine methyl ester (TAME) and the protease inhibitors such as N-tosyl-l-phenylalanine chloromethyl ketone (TPCK), N-α-p-tosyl-l-lysine chloromethyl ketone (TLCK), phenyl methyl sulfonyl fluoride

(PMSF) and soybean trypsin inhibitor type I (SBTI) were purchased from Sigma Chemical Company, USA. The synthetic substrate N-acetyl-l-tyrosine ethyl ester (ATEE) was purchased from CSIR Center for Biochemicals, New Delhi, India. All the other chemicals were of analytical grade and were purchased from local reputed firms in New Delhi.

Extraction and purification of midgut proteases

Penultimate instar larvae were used for enzyme extraction. The larvae were immobilized on ice and dissected out midgut was cut out longitudinally to empty internal food materials as well as the peritrophic membrane. After repeated washings, aliquots of 5 guts were stored at −20°C in 0.5 ml of 0.1 M Tris-HCl buffer (pH 8.0) till further use. The guts along with the buffer were homogenized in a Potter-Elvehjem type of glass tissue homogenizer and the homogenate was centrifuged at 4°C and 5,000 g for 15 min and the supernatant used as crude enzyme solution. The crude enzyme solution was chromatographed on to a glass column (30 × 1.5 cm) of Sephadex G-75 (Pharmacia, Sweden) equlibrated with 0.1 M Tris-HCl, pH 8.0) and eluted with the same buffer at about 5°C. One ml fractions were collected and both the protein content and the protease activity were estimated. Under these chromatographic conditions, total column volume and elution volume were found to be 17 ml and 13 ml respectively.

Estimation of protease activity

Protease activity was determined by the casein digestion method of Kunitz (1947). One % casein stock solution was made by suspending 1g casein ("Hammarsten") in 100 ml of 0.1 M Sorensen's phosphate buffer (pH 7.6). The suspension was heated for 15 min in boiling water to make a complete solution of casein and stored at 10°C in refrigerator.

Samples of 1 ml of 0.5% casein were pipetted out into 15 ml Pyrex test tube and placed in a water bath at 40°C for 5 min before being used. One ml samples of crystalline bovine trypsin dissolved in 0.0025 M HCl were added to samples of 1 ml casein at intervals of about 1 min, mixed well and left at 40°C for 20 min. The solutions were then thoroughly mixed into tubes containing 3 ml of 5% trichloroacetic acid (TCA). The precipitate formed was centrifuged at about 10,000 rpm for 5 min in a clinical centrifuge. Concentration of the proteolytic products in the supernatant solution was determined by measuring the absorbance at 280 nm against a black (1 ml of 0.5% casein, 3 ml of 5% TCA and 1 ml of 0.0025 M HCl). The trypsin concentration and the absorbance at 280 nm were plotted to form a trypsin standard curve after estimating the specific activity (Tryptic Units; T.U.) of the sample of trypsin. For all other experiments, incubation time of 10 min was kept constant.

Parameters of enzymes studied

Estimation of K_m value of purified enzyme fraction using various substrates such as casein, BAEE, TAME, ATEE and BTEE by Lineweaver Burk plot method using KINETICS.BAS package.

Studying the temperature responses of the purified enzyme fractions by allowing the reaction to proceed at different temperature from 30°C to 80°C.

Studying the pH responses of the purified enzyme fractions using casein as the substrate dissolved in buffers of various pH levels.

Trypsin inhibition - The inhibitory activity was studied by measuring the tryptic activity of two samples of the enzyme solutions, one with a definite amount of inhibitor and the other without inhibitor. The difference in tryptic activity of the two samples of enzyme is expressed in tryptic units by referring to the trypsin standard curve plotted according to Kunitz (1947). The inhibitors used in the study were SBTI, TLCK, TPCK and PMSF.

SDS - Polyacrylamide gel electrophoresis (SDS-PAGE) of proteins

The protein profiles of proteases were studied by SDS-PAGE according to the method of Laemmli (1970). A 10% slab gel was cast between the glass plates supplied by Bangalore Genei. A 5% stacking gel (pH 6.8) was also cast over the resolving gel. The protein samples were mixed with an equal volume of 2*x* Laemmli's sample buffer (0.125 M Tris-HCl pH 6.8, 4.6% SDS, 20% sucrose, 10% B - mercaptoethanol and 0.1% bromophenol blue) and boiled for 2-3 min, centrifuged at 10,000 g for 5 min and loaded on to the gel. In each lane the samples containing 100-125 μg protein concentration were loaded. Initially, a current was applied at a constant voltage of 40 V till the samples entered the resolving gel and then increased to 100 V till the completion of run using BIOPRO electrophoresis power pack. Standard protein molecular weight markers were also run in one lane parallel to the samples. The gels were fixed in 12.5% TCA solution for 1 h and stained with 0.25% Coomassie brilliant blue R 250 (Sigma) in methanol-acetic acid-water (40 : 10 : 50) for 4 h and destained using methanol-acetic acid-water (40 : 10 : 50) till the bands were clearly visible.

Determination of molecular weights of proteins

The molecular weights of different bands of protein were calculated by comparing the relative mobility of protein standard markers using QPRO.BAS package.

RESULTS

Protease extraction and purification

The midgut lumen of the test insects when tested with pH paper immediately after dissection revealed the alkalinity; the pH was 9.5 in all the test insects except in *S. litura* whose midgut pH was 10.5.

Among the five species the total protease activity was the highest in *H. armigera* extract (44,500$\times 10^{-4}$ T. U./ml) and was the lowest in *S. litura* extract (19,500 $\times 10^{-4}$ T.U./ml) indicating either higher specific activity or higher purity of the crude extract in *H. armigera* (Table 1).

When the crude extract was chromatographed through Sephadex G-75 and analyzed for protein content as well as protease activity, it was observed that the initial protein peaks had very low protease activity. The protease activity however showed a rising trend after fraction number five and reached a peak at 11th fraction in *H. armigera, A. janata* and *S. litura* and at 13th fraction in the case of *P. xylostella* and *S. obliqua*. The specific activity of the peak fraction was highest (27,666.6 T.U./mg protein/min) in *A. janata* and lowest (6.328.12 T.U./mg protein/min) in *P. xylostella* (Table 1). The results indicated that the protease enzymes can be partially purified by gel filtration technique using Sephadex G-75 which reduced the contamination of high molecular weight proteins. Such purification has increased the specific activity of the

Table 1: Purification of midgut extracts through Sephadex G-75 Chromatography

Source	Crude extract		Purified extract		purification
	total activity	sp. act. (T.U.)/mg.	total activity	sp. act. (T.U.)/mg.	
Achaea janata	33,000	4,230.7	41,500	27,66.7	6.5
Helicoverpa armigera	44,500	6,544.1	61,500	8,124.2	1.2
Plutella xylostella	35,000	3,097.3	40,500	6,328.1	2.1
Spilosoma obliqua	37,500	5,136.9	43,000	13,069.9	2.5
Spodoptera litura	19,500	1,392.8	24,000	11,428.6	8.2

Total activity is in terms of 10^{-4} (T.U.)cas; using 0.5% casein

protease enzymes to many fold. When the specific activities of both crude extracts and partially purified extracts (through Sephadex G-75) are compared, there is a clear indication that the purity of the protease enzyme has increased to about 8.2 fold in *S. litura* extract and to about 1.2 fold in *H. armigera* (Table 1).

Optimum conditions for protease activity

Temperature

The purified midgut extracts showed varying responses when they were assayed for the protease activity using 0.5% casein solution as substrate at different temperatures viz., 30, 40, 50, 60, 70 and 80°C. On the whole the trend was almost similar in the case of *H. armigera, S. litura* and *S. obliqua*. The reaction slowly increased with the corresponding increase in temperature and reached a peak at 60°C in the case of *H. armigera* and *S. litura* and at 50°C in the case of *S. obliqua*. But *A. janata* and *P. xylostella* extracts showed an initial increase in activity followed by gradual and steady decrease in activity at higher temperatures and the peak activity was observed at 40°C and 50°C respectively.

pH studies

Protease assay of the midgut extracts carried out using 0.5% casein solution prepared in 0.1 M Phosphate buffer at various pH of 7.5, 8.0, 9.0, 10.0, 11.0 and 12.0 revealed that the trend was almost similar in *S. litura, P. xylostella* and *S. obliqua* wherein there was an initial increase in the reaction rate which gradually reached a peak at pH 10.0 in *S. litura* and *P. xylostella* and pH 9.0 in *S. obliqua* extract and then gradually decreased. The rate of reaction of *H. armigera* extract increased gradually till pH 10.0 and there was a sudden and steep fall after pH 11.0. *A. janata* extract showed an almost static trend under all the pH conditions studied.

Enzyme Kinetics

The enzyme kinetics were studied under optimum temperatures and pH using casein, ATEE, BTEE, BAEE and TAME. The suitability of the above substrates to the midgut extracts of the test insects was analyzed by estimating the K_m and V_{max} values using Lineweaver-Burk plot. Casein was the best substrate as K_m value was very low (in μ mole lt) with *S. litura* 0.114; *P. xylostella* 0.161; *A. janata* 0.222; *H. armigera* 0.252 and *S. obliqua* 0.259 (Table 2). The affinity of casein for the enzyme in terms of

Table 2: Kinetic constant (K_m) of proteases of different insects

Insect species	Casein (μ mole)	ATEE (m mole)	BAEE (m mole)	BTEE (m mole)	TAME (m mole)
Achaea janata	0.222	1.993	0.634	0.450	0.233
	(0.17)	(0.04)	(0.12)	(0.19)	(0.16)
Helicoverpa armigera	0.252	1.464	3.557	0.230	0.270
	(0.13)	(0.08)	(0.03)	(0.34)	(0.10)
Plutella xylostella	0.161	0.084	4.466	0.289	0.538
	(0.17)	(1.87)	(0.02)	(0.08)	(0.06)
Spilosoma obliqua	0.259	0.162	0.877	0.230	1.126
	(0.12)	(1.06)	(0.08)	(0.38)	(0.05)
Spodoptera litura	0.114	-0.015	1.378	0.162	-0.014
	(0.25)	(-11.5)	(0.05)	(0.52)	(-1.9)

Figures in parenthesis are K values (min^{-1}) based upon V_{max} and K_m; correlation coefficients for different substrates are casein >0.78, ATEE >0.72 except for *P. xylostella* and *S. litura*; BAEE >0.91, BTEE 0.64 except in *S. litura*; TAME 0.51 except *S. litura*.

'K' value as determined by V_{max}/K_m is greater towards *S. litura* (0.25) followed by *A. janata* and *P. xylostella* (0.17), *H. armigera* (0.13) and *S. obliqua* (0.12) midgut extracts. The synthetic substrate ATEE was found not suitable for *S. litura* extract as indicated by negative K_m values. But it was found best suited for *P. xylostella* extract, with K value of 1.87 and for *S. obliqua* that of 1.06. BTEE had the greatest affinity towards *S. litura* 0.114; *P. xylostella* 0.161; *A. janata* 0.222; *H. armigera* 0.252 and *S. obliqua* 0.259 (Table 2). The affinity of casein for the enzyme in terms of 'K' value as determined by V_{max}/K is greater towards *S. litura* (0.25) followed by *A. janata* and *P. xylostella* (0.17), *H. armigera* (0.13) and *S. obliqua* (0.12) midgut extracts.

The synthetic substrate ATEE was found not suitable for *S. litura* extract as indicated by negative K_m values. But it was found best suited for *P. xylostella* extract, with *K* value of 0.52 and the least affinity towards *P. xylostella* extract with K value of 0.08. BAEE showed the greatest affinity towards *A. janata* extract (K value being 0.02). The *S. litura* extract showed a negative trend with TAME as substrate. But the same substrate showed greater affinity towards *A. janata* extract followed by *H. armigera, P. xylostella* and *S. obliqua*.

Protease inhibition

Protease inhibitors such as TPCK, TLCK and PMSF at 10 mM and SBTI at 1% concentration were used in the studied and the purified midgut extracts were assayed for their inhibition of protease activity in the presence of the above mentioned protease inhibitors using 0.5% casein as substrate. Among the inhibitors used PMSF caused a marked reduction in protease activity which is well pronounced in the case of *S. litura* extract (58.62% inhibition) followed by *A. janata* extract (46.43% inhibition). Next to PMSF, SBTI caused a similar inhibition. When trypsin specific inhibitor TLCK and chymotrypsin specific inhibitor TPCK were compared, TLCK has caused a greater inhibition (48.27% in *S. litura* 24.11% in *A. janata*, 11.67% in *P. xylostella*, 11.25% in *S. obliqua* and 9.63% in *H. armigera* midgut extracts) than TPCK (Table 3). The results indicate the chemical nature of purified midgut extracts towards more of trypsin-like

Table 3: Inhibition of protease activity in test insects

Source	Protease activity in 10^{-4} (T. U.) cas with/without inhibitor				
	Control	TPCK 10 mM	TLCK 10 mM	SBTI 1%	PMSF 10 mM
Achaea janata	1120	1120	850	810	600
	(0.00)	(0.00)	(24.11)	(27.68)	(46.43)
Helicoverpa armigera	1350	1290	1220	1660	1120
	(0.00)	(4.44)	(9.63)	(14.07)	(17.04)
Plutella xylostella	1170	1080	975	870	810
	(0.00)	(7.69)	(16.67)	(25.64)	(30.77)
Spilosoma obliqua	1200	1140	1065	870	810
	(0.00)	(5.00)	(11.25)	(27.50)	(32.50)
Spodoptera litura	870	630	450	420	360
	(0.00)	(27.58)	(48.27)	(51.72)	(58.62)

Figures in parentheses are % inhibition of protease activity in relation to control. Protease activity was estimated using 0.5% casein.

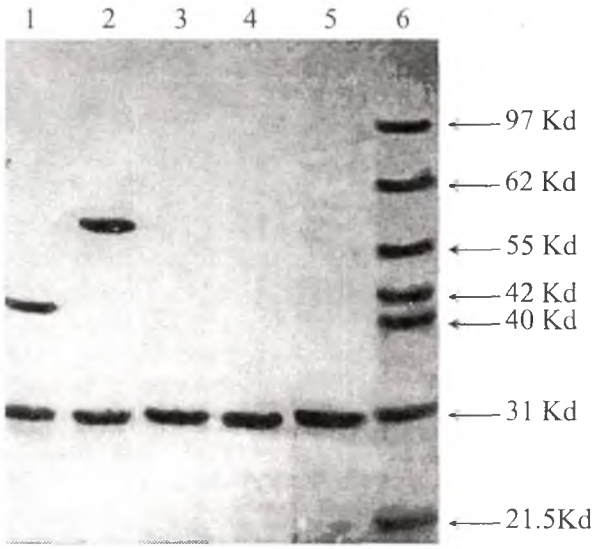

Fig. 1 SDS–PAGE analysis of midgut extracts of test insects purified through Sephadex G–75 Column Chromatography; lane 1-*S. obliqua*, 2-*P. xylostella*, 3-*S. litura*, 4-*A. janata*, 5-*H. armigera* and 6-molecular weight markers.

and to a very limited extent towards chymotrypsin-like nature.

Molecular weights of proteases as determined by SDS-PAGE indicated that *H. armigera* protease has a molecular weight of 30 kd, while that of *A. janata* and *S. litura*, molecular mass was 29 kd each. Both *P. xylostella* and *S. obliqua* midgut extracts had two protein bands each with molecular weights of 56 and 29; and 40 and 29 kd respectively (Fig.1).

DISCUSSION

It is evident from the above results that the alkaline midgut region of the test insects is conducive to proteases to degrade efficiently the proteinaceous substrates present in the food to derive the required aminoacids after digestion for the growth and development. This observation has already been confirmed in insects belonging to various families of Lepidoptera (Miller, 1974; Ahmad et al, 1980; Pritchett et al, 1981; Sasaki and Suzuki, 1982; Euguchi and Kuriyama, 1985; Broadway, 1989; Teo et al, 1990).

Gel filtration of the crude midgut enzyme extracts has eliminated the contaminating small molecular weight proteins and other substances that are usually present in the cruse extracts and increased the purity of the enzyme. Such purification has in turn increased the specific activity of the protease enzymes many fold (Ahmad et al, 1980; Srivastava, 1988; Johnston et al, 1991). The purified midgut extracts of all the test insects required an optimum pH range of about 9.5–10.0, an optimum temperature range of about 40-60°C and a reaction time of 10 min for effective proteolysis of casein. These results were similar to those reported for *Trichoplusia ni* (Pritchett et al, 1981) and *S. litura* (Ahmad et al, 1980; Srivastava, 1988). The caseinolytic activities and the hydrolysis of the synthetic substrate BAEE indicate that the major proteolytic activity of the midgut extracts is mainly due to a trypsin-like fraction which is in agreement with earlier findings (Ahmad et al, 1980; Miller, 1974; Law et al, 1977; Purcell et al, 1992). The protease inhibitor studies using various inhibitors indicate trypsin-like nature of purified midgut extracts of the test insects. A greater inhibition by PMSF, SBTI and TLCK indicates the essential role of a serine and a histidine residue in the endopeptidase enzyme activity (Shaw et al, 1965; Christeller and Shaw, 1989; Lenz et al, 1991; Christeller et al, 1992).

The molecular weights of the purified midgut extracts determined by SDS-PAGE analysis are between 29 and 31 kd similar to that of some lepidopterous midgut proteases (Ahmad et al, 1980; Miller, 1974; Johnston et al, 1991). Molecular weight of protease of *H. armigera* was about 24 kd by SDS-PAGE and gel filtration studies (Johnston et al, 1991). Ahmad et al (1980) reported three proteases with molecular weights of 17, 21 and 53 kd in *S. litura* while our studies showed only one of 29.29 kd in the elute in 13-15 from Sephadex, although a peak of protease activity could be seen in elute 3-5 that could be high molecular weight protease like that reported by Ahmad et al (1980).

It is evident from the present work that the test insects possess alkaline proteases in the midgut region which are almost having similar properties with respect to the optimum conditions of pH, temperature, time and differing in their substrate and inhibitor specificity which probably make the insects to thrive on certain selected host plants utilizing different plant proteins quantitatively *vis-a-vis* abundance and survival of herbivore insects.

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Effect of Diet on the Survival of the Blowfly, *Chrysomya chloropyga* (Wied.) (Diptera: Calliphoridae) Under Laboratory Condition.

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Abstract: *Chrysomya chloropyga* Wiedemann is a cosmopolitan species and a causative agent of cutaneous myiasis in sheep and cattle. The survival of *C. chloropyga* was investigated by maintaining laboratory-reared adult males and females in illuminated cages using three different diets, comprising (a) mixture of ground rice and fish, and sugar (b) mixture of ground rice and fish (c) sugar only, at temp. $28 \pm 2^{\circ}C$ and $75 \pm 5\%$ R.H. water was provided in all experiments. Survival of males and females of *C. chloropyga* maintained together or in isolation on a mixture of ground rice and fish, and sugar was significantly prolonged than those maintained on a mixture of ground rice and fish, and sugar only. Males and females on a mixture of ground rice and fish lived longer than those on starvation but significantly lower than those maintained on sugar only. Optimal survival was obtained when males and females were maintained on sugar only and when fed on a mixture of ground rice and fish, and sugar.

INTRODUCTION

Insect survival and development are affected by several environmental factors including temperature as well as nutritional factors (Vrba *et al.*, 1983; Lysyk, 1991). Liles and De long (1960) showed that mated females of *Aedes aegypti* lived longer than virgin when caged alone than together with females. Nayar and Sauerman (1971) investigated the influence of diet on the life span of *A. taeniorhynchus* and reported that females fed on protein and no sugar lived significantly shorter than sugar-fed ones. Briegel and Kaiser (1973) reported optimal survival for males of *A. aegypti* and *Culex pipiens fatigans* fed on sugar and females on sugar and protein. Lysyk (1991) demonstrated that milk promotes longevity in *Musca domestica*. *Drosophila melanogaster* adults fed on glucose lived longer than when given yeast or caesin (Hollingsworth and Burcombe, 1970). Adult female *A. aegypti* fed aqueous extracts of bee-collected pollen lived longer than those fed on 10% sucrose (Eischen and Foster, 1983).

Chrysomya chloropyga is a bluish-green blowfly found in most parts of the tropical world. Under natural condition, it feeds on carrion, visits refuse bins and feed on decomposing wastes. *C. chloropyga* feed on exposed wounds of sheep and cattle, causing

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Table 1: Sex and maintenance diets.

Sex	Diet
Males and females together	Mixture of ground rice, fish and water, and sugar.
Males only	same
Females only	same
Males and females together	Sugar only
Males only	same
Females only	same
Males and females together	Mixture of ground rice, fish and water.
Males and females	Starvation (water only)

myiasis in these animals. The effect of diet on the longevity of of *C. chloropyga* has not been investigated and in spite of its ubiquity in Nigeria, knowledge of its biology is very scanty (Muse and Balogun, 1986). The purpose of the present investigation was to determine the effect of diets on the survival of adult *C. chloropyga* under artificial conditions in the laboratory.

MATERIALS AND METHODS

Adult *C. chloropyga* were reared and maintained in an illuminated cage (60w bulb) measuring 45 × 30 × 30cm at temp. 28 ± 2°C and 75 ± 5% R.H. The rearing medium was a mixture of ground rice, ground fish and water (1 : 1 : 1 : 5 w/v) and sugar (Anantiko *et al.*, 1982). Water was provided in a petri dish soaked with cotton wick in all cages. Within 24h following emergence, adult male and female *C. chloropyga* were provided with food according to Anantiko *et al.*(loc. cit.) with modification as shown in Table 1

The nutrient media were regularly checked for eggs which were removed as they were laid. The media were also changed every 72h, except sugar which were replaced when exhausted. From the first day of maintenance of flies on the different media, dead flies were picked and recorded for as long as they lived. The data obtained were used to determine mean survival time (MST), 50% survival time (50% ST) and maximum life span. The data was also analyzed using student t-test and Analysis of Variance test

RESULTS

The mean survival time (MST), 50% survival time (50% ST) and maximal life span in days have been determined as shown in Table 2. Males and females maintained on a mixture of ground rice and fish, and sugar had maximal life span of 68 and 66 days respectively. 90% survival was obtained for males and females up to 35 days of age and 50% ST were 48.7 and 45.3 days for males and females respectively. Males maintained in isolation on a mixture of ground rice and fish, and sugar separately lived significantly longer than females similarly maintained ($t = 7.5$; df $= 2$, $p < 0.05$) but there was no significant difference in the MST of males and females maintained together on the same diet.

Males and females maintained together on sugar only showed a lower MST for males in relation to females ($t = 22.6$; df $= 2$; $p < 0.05$). 50% ST was also longer

Table 2: Survival data for male and female *C. chloropyga* on different diets.

Diet	Sex	Number of flies	MST, Mean S.E. (days)	50%ST mean (days)	Maximum life span (days)
Ground rice and fish, and sugar	(M) and (F)	66	61.7 ± 2.34	48.7	68
		63	65.7 ± 1.45	45.3	66
Ground rice and fish, and sugar	(M)	42	38.7 ± 1.45	31.3	41
Ground rice and fish, and sugar	(F)	33	23.7 ± 3.39	10.7	26
Sugar only	(M) and (F)	37	26.3 ± 1.76	14.7	29
		22	35.0 ± 1.73	19.7	38
Sugar only	(M)	16	27.7 ± 2.03	10.0	31
Sugar only	(F)	28	19.7 ± 1.45	17.3	22
Ground rice and fish	(M) and (F)	33	7.3 ± 0.67	7.0	8
		55	8.7 ± 1.45	7.3	11
Starvation (water only)	(M) and (F)	27	3.3 ± 0.33	3.0	4
		25	3.3 ± 0.33	2.3	4

for females. However, males maintained in isolation had a longer and significant MST than females similarly maintained ($t = 13.8$; df $= 2$; $p < 0.05$). Maximal life span of males was 31 days and females 22 days but 50% ST was longer for females. There was no significant difference in the MST of males maintained with females and males maintained in isolation on sugar. Females similarly maintained with males

lived significantly longer than females maintained in isolation ($t = 4.50$; df $= 2$; $p < 0.05$).

The mean survival time (MST) for males and females maintained together on a mixture of ground rice and fish was significantly lower than MST of males and females maintained together on a mixture of ground rice and fish, and sugar respectively ($t = 11.34$; df $= 2$; $p < (0.05)$ ($t = 57.0$; df $= 2$; $p < 0.05$). 50% ST of 7.0 and 7.3 days for males and females respectively were comparatively lower and close to their maxima life span. Males and females exposed to starvation had maximum life span of 4 days. There was no significant difference in their 50% ST, MST and maximal life span.

There was a significant difference in the MST of males exposed to different diets ($F = 177.4$; df $= 5, 12$; $p < 0.001$). MST of females similarly treated were also significant ($F = 144.5$; df $= 5, 12$; $p < 0.001$). There was no significant difference in the MST ($f = 0.01$; df $= 1, 10$; $p < 0.001$) and maximal life span ($F = 0.03$; df $= 1, 10$; $p < 0.001$) of males and females exposed to different diets but males and females exposed to sugar only survived longer than males an females maintained on a mixture of ground rice and fish only ($F = 101.6$; df $= 1, 4$; $p < 0.001$) ($F = 135.4$; df $= 1, 4$; $p < 0.001$).

DISCUSSION

The survival of males and females of *C. chlropyga* was studied under different feeding conditions in the laboratory. Maximal life span of 68 days for males and 66 days or females was obtained when the flies were fed with a mixture of ground rice and fish, and sugar. The life span were significantly different from those obtained on other diets suggesting the suitability of the diet which contain protein and carbohydrate for maximum longevity. Nayar and Sauerman (1971) reported maximal life span of 100 days for females and 60 days for males of *A. taeniorhynchus* fed with sugar and blood. There was significant difference in the length of life of virgin and mated *C. chloropyga* as reported for *Drosophila* species (Collatz and Hoeger, 1980). MST of females maintained in isolation either on a mixture of ground rice and fish, and sugar or sugar only were rather low. This suggests that female longevity is enhanced by the presence of males. Male material transferred to female butterfly seem to have positive effect on their longevity (Boggs and Gilbert, 1979). Females require protein food, in addition to sugar for better survival since males survived longer than females when both were maintained in isolation on sugar. Maximum longevity of 29 and 38 days for males and females *C. chloropyga* respectively is comparable to those of horse flies maintained on sugar (Wilson, 1967).

males and females *C. chloropyga* reared on sugar lived significantly longer than males and females maintained on a mixture of ground rice and fish without sugar. Lysyk (1991) suggested that absence of feeding on sucrose may be a factor determining housefly longevity. It seems sucrose (disaccharide) is readily hydrolyzed to glucose which provide energy in comparison to ground rice (polysaccharide). The deprivation of male and female *C. chloropyga* of sugar strongly affect their longevity since maximum life span of males and females deprived of sugar was significantly shorter than those with sugar in their diets. Lysyk (1991) reported that houseflies fed sucrose lived for 28 days while those without it lived for 7 days. *A. aegypti* and *Culex pipiens fatigans* fed protein but deprived of sugar lived longer than those on starvation but significantly shorter than sugar fed one (Briegel and Kaiser, 1973)

Newly emerged male and female *C. chloropyga* on starvation survived for 4 days. Van Handel and Lum (1961) reported that houseflies consume residual fat from pupal stages but the energy reserves accumulated in larval stage of *A. sollicitans* is sufficient to maintain the adults for several days (Vam Handel, 1984). *C. chloropyga* adults on starvation after eclsion probably survives on residual energy reserves acquired from the larval and pupal stages which is depleted after 4 days of adult life.

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Water Storage and *Aedes aegypti* Breeding in Multistoreyed Flats: a Case Study in Pune, Maharashtra State, India

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Abstract: Water storage and *Aedes aegypti* mosquito breeding was examined in flats at Lokmanya Nagar, Pune an area with three Storeyed buildings. Two surveys were conducted in 1983 and 1987, when stable breteau indices (BI) of 12.21 and 11.58 respectively were observed. In storey III *Ae. aegypti* prevalence was low (BI=$\sim$ 5.0) and the volumes of water stored increased apparently due to water shortage. In storeys I & II the BI was >10.0 during both the surveys. The difference in the number of water storage containers between storey I and II was nonsignificant. An attempt to explain these results by inductive deductions of expected patterns using combinatorial analysis was made. Significance of these results were examined in the wake of: (a) increasing multistoreyed housings, (b) ensuing water shortage and (c) the incidence of dengue fever in the state. It is felt that the cost of *Ae. aegypti* control in such areas could be optimised by emphasising the source reduction in lower storeys.

Keywords: *Aedes aegypti* mosquito breeding containers, water shortage, flats, multistoreyed buildings.

INTRODUCTION

Dengue is by far the most important arbovirus infection in the South-east Asia and poses threat to $\sim$2 billion people in over 100 countries world wide. Dengue fever (DF) is a mild disease but the severe form — dengue haemorrhagic fever (DHF) causes considerable morbidity and mortality. The rise of DF/DHF is explained by the factors such as rapid population growth, expanding urbanisation, inadequate water supplies and difficulties in refuse disposal (Lam, 1998).

Rapid urban expansion has resulted in a considerable stress on already meager drinking water supplies. These supplies were observed to reach the top storeys with difficulty (Macdonald, 1956). This might lead to a gradient of water shortage across storeys and as a consequence, to variegated water storage practices.

The principal vector mosquito of DF/DHF– *Aedes aegypti*(L.) (Diptera: Culicidae) breeds in the artificial containers either used for water storage or discarded junk which

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gets rain filled. The nature and numbers of water storage containers differ with water supply and cultural practices in different ethnic societies (Macdonald, 1956; Pichon *et al.*, 1969; Barrera *et al.*, 1993). But the assessment of their impacts on *Ae aegypti* populations, are necessary. In Singapore the rate of *Ae. aegypti* establishment in flats was estimated to account for annual variation following their inhabitation (Chan *et al.*, 1971). Subsequent to an epidemic of dengue in 1982 in Delhi, *Ae. aegypti* breeding was observed in a number of overhead tanks (OHT) (Uprety *et al.*, 1983); and in Kuala Lumpur *Ae aegypti* was recorded on all the storeys including the roof tops (Sulaiman *et al.*, 1993).

A case study aids in a significant contribution to a disease or disease group and aims to collect useful information for transdiseases areas (UNDP/WHO/TDR, 1996). Thus water storage containers which support *Ae. aegypti* breeding could also harbour parasitic contaminants of water borne diarrhoeas (Jonnalagadda and Bhat, 1995). Present case study reports *Ae. aegypti* breeding pattern in water storage containers in three storeyed flats in Pune and envisages the relevance of increasing multistoreyed buildings and their impact on the cost of *Ae. aegypti* surveillance and control.

METHODS OF SURVEY

Study area

Ae. aegypti reappeared in Pune city in 1970s (Geevarghese *et al.*, 1975). Lokmanya Nagar, Pune a three storeyed residential area had a history of *Ae. aegypti* infestation with breteau index (BI)- 4.76 in June 1971 and nil in January 1972 (Mahadev *et al.*, 1978). Developed in 1960s, it has 777 flats in 51 buildings; flats are of two types: A-type with 31.12 sq.m. carpet area, and B-type with 27.13 sq.m. The ratio of A&B types was 40.92:59.08. The municipal piped water supply was redistributed within these buildings by separate overhead tanks for potable ('p'=drinking & cooking) and non-potable ('np'=washing and bathing) purposes.

Surveys

One larva-per-container survey method was used (Sheppard *et al.*, 1969) with a data sheet per flat to collect: (i) number of containers with water, (ii) volume of water stored (only in 1983) and (iii) *Ae. aegypti* breeding in them. The volume classes of the containers were identified in 1987 based on 1983 experience.

The first 21 buildings were surveyed in 1983 and the entire 51 buildings in 1987. Hot dry months of April & May were chosen during both visits since: a) the *Ae. aegypti* productivity of water storage containers peaked during these months (Reuben, *et al.*, 1978) the virtual absence of other water-container competing *Aedes* spp. that could affect the relative prevalence assessments by sharing of larval habitats (Mahadev *et al.*, 1978; Service, 1974). At least one flat on each storey was visited in 1983 and all the flats in 51 buildings in 1987; visits were timed between 08.00 to 11.30 hrs.; flats on the ground, middle and top storeys were numbered I, II and III respectively for convenience.

Assumptions

Prerequisite criteria a habitat study needed to fulfill were: a) Uniform sampling unit and b) stability of the populations in question (Southwood,1977,1978). Plinths of the

flats (this study) and the seasonal stability of Breteau indices (BI=number of larval habitats per 100 houses) in the residential areas (Soman, 1977) were assumed to serve both the criteria. BI was also shown to represent *Ae. aegypti* populations more closely than the container index (CI) or the house index (HI) (Chan *et al.*, 1971).

Analysis

The elements of present survey are flats [=Houses(H)], water holding containers (N) and *Ae aegypti* breeding containers (N). The interrelationships between the component sets are as follows[1]:
N^+ C N; Nnp^+ C Nnp; Nnp^+ C D (drum)
N=Nnp U Np; and
Np^+ C Np; as a consequence
CI. (Nnp) > CI.(N).

Recounting the present study as a random experiment and expecting that increasing, decreasing or stable conditions of N^+ and N, the total number of predictable outcomes (sample space) would be $(3)^2 = 9$ (Fig. 1) (Hogg and Craig, 1970). Three data points in H domains denote storeys thus increasing the sample space to $3^2 \times 3^2 = 81$ (Fig. 2). Homogeneity of H & N were tested by χ^2 tests for small samples of poisson data; Storey wise average volume of water stored was compared using students 't' test (Bailey, 1959). Storey to storey comparisons of H^+/H^- or N^+N^- from the survey data were performed by $2 \times 2 \cdot \chi^2$ tests and odds. ratios (OR) (Dean *et al.*, 1990).

RESULTS

1983 survey

Among 179 flats in 21 buildings canvassed 5.59% residents refused permission, 21.33% were locked; Out of 131 flats surveyed, the total H between storeys did not differ within A type ($\text{Av.H} = 20, \chi^2 = 0.4, \text{d.f} = 2, p > 0.05$) and within B type ($\text{Av.H} = 23.67, \chi^2 = 0.4506, \text{df} = 2, p > 0.05$) (Table 1). The proportions of flats surveyed did not differ by type ($\text{A} : 60/318; \text{B} : 71/459; \chi^2 = 1.55, p > 0.05$). The number N differed between storeys among A type ($\chi^2 = 9.07, \text{df} = 2, p < 0.05$) but nonsignificant among B type ($\chi^2 = 3.137, \text{df} = 2, p > 0.05$); differences among Nnp ($\text{A} : \chi^2 = 4.33; \text{B} : \chi^2 = 1.7639$) and of "D" ($\text{A} : \chi^2 = 4.408; \text{B} : \chi^2 = 1.8568$) were nonsignificant ($p > 0.05$). Thus the difference in N among A type owed to the merger of the set Np.

Nnp volume per flat increased significantly between storey I & III ($t = 3.31; p < 0.05$) and II Vs III ($t = 2.38; p < 0.05$) in A type; but without statistical significance between I & III ($t = 1.01; p > 0.05$) and between II & III ($t = 0.92; p > 0.05$) in B type flats; difference between storeys I & II was always nonsignificant. Np volume did not differ between storeys significantly; differences between storeys in total volume (N=Np+Nnp) was nonsignificant consistently either for A or B types. A UB flats there was nonsignificant difference in N volume (Np U Nnp) between storeys I & II, but the differences between II & III or I & III were considerable though not significant. The flatwise total volume of water stored was significant for Nnp > Np.

[1] N^+CN- reads as N^+ is contained in N; U-union of sets

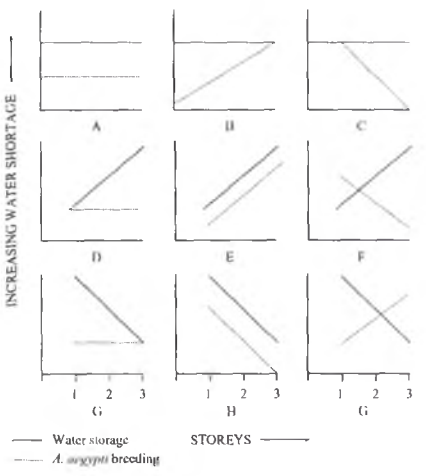

Fig. 1: Hypothetical expectations of *A. aegypti* breeding in multistoreyed buildings.

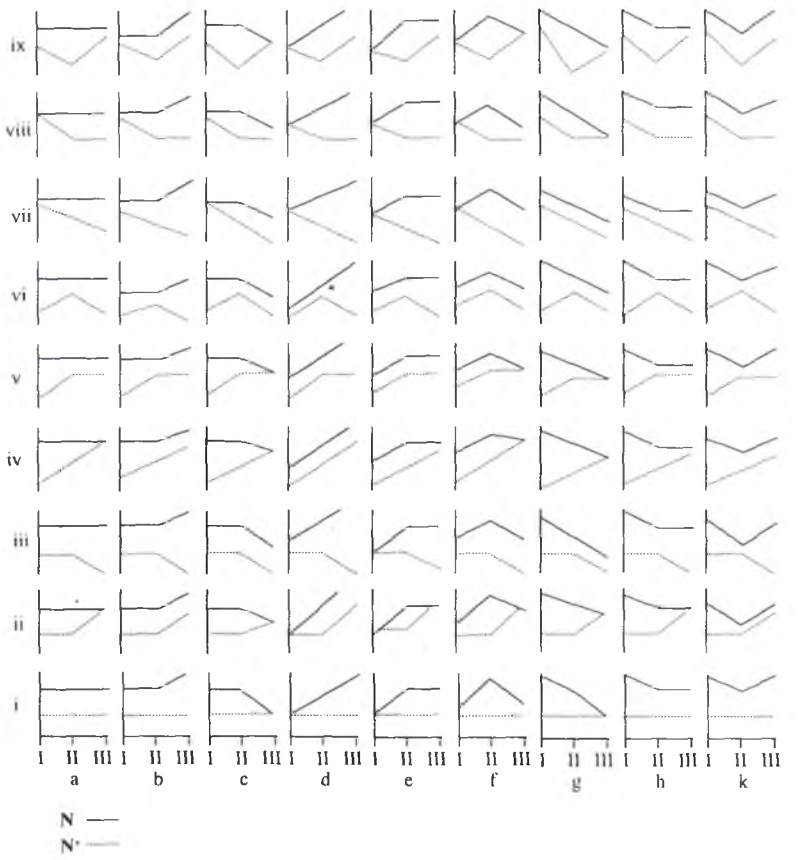

Fig. 2: Schematic representation of the sample space of the water containers available (N) and those of positives (N^+) (i – ix) in relation to storey height (a – k).

Table 1: Summary of water storage practices and *Ae. aegypti* breeding in Lokmanya Nagar, Pune, 1983.

Type	Storey	H	H^+	N^+/N				Volume of water stored			BI
				D^+/D	N^+/Nnp	Np^+/Np	N^+/N	Nnp	Np	N	
	I	22	3	0/10	3/36	0/39	3/75	2295.26	815.1	3110.36	12.64
A	II	18	1	0/13	1/25	0/22	1/47	2333.7	513	2846.7	5.56
	III	20	3	1/21	3/42	0/37	3/79	4444.0	656	5100.0	15.00
	I	25	5	6/20	6/27	0/37	6/64	3041	685	3726	24.00
B	II	21	3	3/14	3/20	0/27	3/47	2578.59	611.1	3189.69	14.29
	III	25	0	0/22	0/29	0/34	0/63	3917.25	712	4629.25	00.00

Note: N^+,N – Number of containers with water +ve for *Ae. aegypti*, numbers examined;
$Nnp^+(=N^+)$,Nnp – Number of containers with water for nonpotable purposes +ve for *Ae. aegypti*, numbers examined;
D^+,D – Number of iron Drums with water +ve for *Ae. aegypti*, numbers examined;
H^+,H – Number of Households +ve for *Ae. aegypti*, number examined.

Pattern of *Ae aegypti* breeding-1983 data

The overall BI was = 12.21; total number N^+ were small but they were 9, 4 and 3 in storeys I, II & III respectively; storeywise distribution of ornamental plant/flower vases among the N was 45.45%, 21.21% and 33.33%; and their N^+ were 13.33%, nil and 18.81% respectively. On exclusion of these poorly productive containers, distribution of N^+ was 7, 4 and 1 in respective storeys thus declining with increasing storey height. Overall patterns of Nnp, Nnp^+ or D, D^+ across storeys were similar to N, N^+ (Fig. 2, vii.k); However A type flats exhibits ix.k pattern and the N by volume showed storeywise increase (Fig. 2).

1987 survey

Homogeneity of data and Container distributions: Of 774 flats canvassed, 1.42% refused permission, 22.35% were locked and 2.59% of the rest had $N = 0$. The numbers of H did not differ between storeys either within A type (Av.H = 79.33, df = 2, $\chi^2 = 0.2605$, $p > 0.05$) or B type (Av.H = 110.67, df = 2, $\chi^2 = 0.1145$, $p > 0.05$). the ratio of A & B type flats surveyed was 41.75 : 58.24($n = 570$) difference between them was nonsignificant ($\chi^2 = 0.61$; $p > 0.05$). While N were different between the storeys among A type (Av.N = 190.33, df = 2, $\chi^2 = 9.145$, $p < 0.05$), the difference was nonsignificant among B type (Av.N = 390.33, df = 2, $p > 0.05$); within A type differences were significant among Nnp (Av.Nnp = 107.67, df = 2, $\chi^2 = 9.8511$, $p < 0.01$).

The Nnp proportions either between storeys I and II or within A & B types of flats did not differ significantly; but differed significantly between type of flats within storey III. This owes to a proportionate rise in the Np in B type which, contributed little to the N^+. In both the surveys $N^+p = 0$; hence $N^+ = N^+np$. (Table 2 & 3).

Pattern of H^+, N^+ & Nnp^+ - 1987 data

Overall N^+ was sparse and BI = 11.58 In all 75 paired comparisons were performed to study differences between and/or within storeys of the factors H^+/H, N^+/N, Nnp^+/Nnp and D^+/D.35 among these were tests of the above factors between A vs. B flats on

Table 2: Summary of *Ae. aegypti* collections in Lokmanya Nagar, Pune, 1987.

Type	Storey	H	H^+	D^+/D	Nnp^+/Nnp	Np^+/Np	N^+/N	BI
	I	77	9	5/40	11/102	0/88	11/190	14.29
A	II	78	12	11/39	13/88	0/73	13/161	16.67
	III	83	4	3/60	4/133	0/87	4/220	4.82
	I	108	14	12/66	15/188	0/191	15/379	13.89
B	II	111	16	15/72	17/195	0/210	17/396	15.32
	III	113	6	6/82	6/193	0/204	6/397	5.31

Note: Letters on columnheads donote same as in table 1.

each storey; they were nonsignificant ($p > 0.05$). So combined data for A & B flats was used for the remainder which also avoided empty cells in $2 \times 2\chi^2$ tests (Table 3).

The tests for difference among N^+/N between storeys Ivs.II were nonsignificant; the χ^2 values increased in all Ivs.III and IIvs.III comparisons; among these, χ^2 in Ivs.III were significant or marginally nonsignificant; those of IIvs.III were different at $p < 0.05/ < 0.01$. The rate of H^+, N^+, Nnp^+ and D^+ in storey III was consistently lower than storey II during both the surveys (Table 3). Irrespective of the significance level shown in many comparisons the respective ORs ≤ 1 N^+/N in storey III; storey I/ II/ I&II were >2.

N,N^+, NNp,Nnp^+ and D,D^+ distribution patterns resembled iii.k for A type flats; iii.e for B type and the combined data. However when the denominator was 100 for N & N^+ they resembled vi.k, iii.a and iii.k for A, B types and total flats respectively (Fig. 2).

Habitats

The total N were 2118 and N^+, 83 (Table 4). The summary included (not in tables 1 & 2): (a) uncovered and/or ill maintained 28 OHT on the rooftops but $N^+ = 0$ and (b) outdoor containers outside storey I but within the domestic ambit; (c) vessels and sundry in the balconys of storeys II & III, may have been filled during early showers. The OHT on remaining buildings were covered and locked; some synthetic/cement tanks or modified metal drums were situated on the atticks served as secondary supply tanks; being well covered, they were not examined (Table 4). The volume classes of N in 1987 and 1983 were similar. Containers of larger volume -*e.g.* plastic barrels of >75 litres etc., noted during 1987 study were included in miscellaneous ones in 1983.

DISCUSSION

Distribution

Since all the single *Aedes* larvae collected at Lokmanya-Nagar were *Ae aegypti*, BI represented its populations; secondly equable BI in replicate surveys also were comparable with those in Bangkok (Tonn and Bang, 1971). *Ae aegypti* distribution in multistoreyed buildings within Pune Urban agglomeration was variegated e.g., (a) Sahakar Nagar (off Pune-Bangalore highway) had BI < 5.0; (b) Aggarwal colony had

Table 3: $2 \times \chi^2$ and Odds Ratios (OR) comparisons of A∪B apartment types

Storeys	Factor	χ^2	p	OR	95% C.L.
1983 data					
I vs. II	H^+ vs. H^-	0.35	>0.05	1.79	0.44, 7.86
II vs. III	"	0.04	>0.05	1.6	0.28, 9.81
I vs. III	"	1.46	>0.05	2.87	0.63, 14.85
I∪II vs. III	"	1.55	>0.05	2.27	0.55, 10.79
I vs. II ∪ III	"	0.33	>0.05	1.38	0.41, 4.75
I vs. II	N^+ vs. N^-	0.99	>0.05	1.56	0.42, 6.22
II vs. III	"	0.31	>0.05	2.06	0.38, 11.98
I vs. III	"	2.29	>0.05	3.21	0.77, 15.3
I∪II vs. III	"	2.6	>0.05	2.74	0.71, 12.32
I vs. II ∪ III	"	2.69	>0.05	2.26	0.75, 6.93
I vs. II	Nnp^+ vs. Nnp^-	0.3	>0.05	1.71	0.44, 7.15
II vs. III	"	0.39	>0.05	2.21	0.39, 13.25
I vs. III	"	4.14	<0.05	3.78	0.88, 18.61
I∪II vs. III	"	3.21	>0.05	3.1	0.71, 14.3
I vs. II ∪ III	"	3.41	>0.05	2.6	0.83, 8.25
I vs. II	D^+ vs. D^-	0.31	>0.05	2.0	0.38, 11.62
II vs. III	"	1.03	>0.05	5.25	0.44, 138.92
I vs. III	"	4.49	<0.05	10.5	1.12, 245.67
I vs. II ∪ III	"	3.71	>0.05	4.13	0.92, 19.42
I∪II vs. III	"	3.55	>0.05	7.88	0.95, 172.82
1987 data					
I vs. II	H^+ vs. H^-	0.27	>0.05	0.85	0.43, 1.54
II vs. III	"	10.2	<0.01	3.23	1.45, 7.34
I vs. III	"	6.46	<0.05	2.64	1.16, 6.14
I∪II vs. III	"	0.64	>0.05	1.3	0.72, 2.32
I vs. II ∪ III	"	9.8	<0.01	2.4	1.4, 6.32
I vs. II	N^+ vs. N^-	0.32	>0.05	0.86	0.48, 1.52
II vs. III	"	12.19	<0.001	3.26	1.61,6.62
I vs. III	"	8.74	<0.01	2.91	1.33, 6.51
I∪II vs. III	"	12.08	<0.001	3.51	1.54, 6.62
I vs. II ∪ III	"	1.5	>0.05	1.37	0.8, 2.23
I vs. II	Nnp^+ vs. Nnp^-	0.43	>0.05	0.83	0.46, 1.49
II vs. III	"	14.01	<0.001	3.75	1.72, 8.36
I vs. III	"	9.7	<0.01	3.11	1.41, 7.04
I∪II vs. III	"	13.73	<0.001	3.42	1.66, 7.25
I vs. II ∪ III	"	1.66	<0.05	1.4	0.81, 2.41
I vs. II	D^+ vs. D^-	1.86	>0.05	0.62	0.3, 1.3
II vs. III	"	15.26	<0.001	4.52	1.91, 10.98
I vs. III	"	6.08	<0.05	2.82	1.13, 7.21
I∪II vs. III	"	12.19	<0.001	3.65	1.64, 8.36
I vs. II ∪ III	"	0.29	>0.05	1.19	0.6, 2.33

high BI > 20.0; (c) Gurunanak nagar along Pune-Solapur highway in Pune, Indrayani-darshan and Ashok-nagar, Dehu Rd. town had BI = 0 (Mahadev *et al.*, 1978, Mahadev, 1983, 1985). Similarly variegated records of *Ae aegypti* in flats followed the DF outbreaks: e.g., (a) new flats in Sangli (Maharashtra), within 3/4 km. of a focal outbreak of DF at Miraj, were negative in 1985 (MDG. Unpubl. data); (b) the sole denge virus

Table 4: Distribution of *Ae. aegypti* +ve/examined habitats by volume class

Type of Habitat	Volume class (litre)	A-Flats I	A-Flats II	A-Flats III	A-Flats Total	B-Flats I	B-Flats II	B-Flats III	B-Flats Total
1983 Data									
Drums	70-250	0/12	0/13	1/23	1/48	6/21	1/18	0/27	7/66
Vessels	10-30	2/32	0/24	1/31	3/77	0/37	0/25	0/39	0/101
Vases	1≥	2/14	3/14	0/16	5/44	0/6	0/5	0/2	0/13
Cement tanks	100≤	1/1	0	0/1	1/2	-	-	-	0
1987 Data									
Drum	70-250	5/40	11/39	3/60	19/139	12/66	15/72	6/82	33/220
Vessels	10-30	0/128	0/92	1/122	1/342	0/241	0/251	0/251	0/743
Vases	1≥	5/53	2/28	0/60	7/141	2/64	2/68	0/56	4/188
Cement tanks	100≤	1/3	0/2	0/2	1/7	1/8	0/5	0/8	1/21

antigen positive female *Ae aegypti* was collected from a flat on storey II at Dhanilimda, Ahmedabad city (Gujarat); its breeding could be detected only in an adjoining slum and a close set housing (BI = 23.52; $n = 34$); On the other hand in the multistoreyed slums at Milattnagar BI was 42.86 ($n = 7$) and at the posch flats at Tavadipura (Behrampura) BI was 30.00 ($n = 20$) (Recalculated from (Mahadev *et al.*, 1993). Since its adult presence signifies a potential disease risk (Anonymous, 1986) *Ae aegypti* prevalence at the Parvathi-foothill slum adjoining Lokamanya Nagar needs attention should a control programme be envisaged (MDG unpubl. data, 1982–87).

Upsurge of Multistoreyed housings, *Ae aegypti* surveillance costs

The multistoreyed buildings are increasing in numbers and height in small and large cities alike. The upsurge of new townships in the coastal region of Maharashtra during 1980s and 90s are marked by many a multistoreyed housing at New-Mumbai (Bombay), New-Panvel, residential areas Jawaharlal Nehru Port Trust (JNPT; Raigadh district). *Ae aegypti* breeding was noted only in tyres adjoining some flats at Kalamboli (New-Panvel) but not at the JNPT (Unpublished data, 1995). Interestingly when such housing is inhabited anew, water storage was observed to be either absent or minimal; and so was *Ae aegypti* adult incidence/breeding absent.

The flats represented posch/affluent residentia in small towns (*i.e.*, <49,999 population). In the cities of Pune and Ahmedabad on the other hand, array of flat types range from the slums to luxury bungalows occupying vertical space. Not withstanding the height of these structures, the costs of *Ae aegypti* larval surveys increase with the size class of the flats. The survey costs in terms of area/effort ranged from ~20–30 houses/man-hr. in slums/hutment areas, 10–20 houses/man-hr. in middle class housing to ~5.0 bungalows/man-hr. (estimated from Mahadev *et al.* 1978). Viewing the flats sampled within this cost-framework, Lokamanya Nagar represents a middle class housing.

Closed houses and nonrespondents which affect vector control programmes (Moser

and Kalton, 1971; Chadee, 1988) accounted for >26% houses canvassed in this study. Repeat visits required to cover the missing houses would add to the sampling costs. To remedy this and to optimise the efforts, (a) timing of larval collections early in the day, (b) coincidence with a weekend, (c) an industrial holiday or even a school vacation were suggested. While 'a' was adopted in this study 'b & c' could be adopted during DF/DHF epidemic emergencies.

Disposition

It was difficult to explain the observed patterns using a simple model of either *Ae aegypti*'s +ve/-ve geotropy and by using BI/CI. 1983 collections showed that the breakdown of elements of survey and perceived factors were inadequate since storey height increased twice in three storeyed environment; this multiplied the sample space twice. Thus an increase in sample space by 3^2 for every rise by one storey can be expected. This extended model magnified the scope to falsify either causes viz., (i) positive geotropy of the ovipositing *Ae aegypti* contributing to the increasing breeding in lower storeys; (ii) the water shortage dependant increases in water shortage consequent to increases in storey-height and consequent impacts of the incident factors.

1983 data supports the causality of positive geotropic disposition by *Ae aegypti*. Its increased breeding in storey II during 1987 survey was intriguing which was also seen in Kuala Lumpur (Sulaiman *et al.*, 1993). The differences among storey I & II might owe to its transitory breeding (Windeguth *et al.*, 1969).

The size and number of N may be regulated by diverse factors e.g., (a) **size of the house** – the nonsignificant difference in N between A and B type flats on each storey indicated that the segregation of the type of flats by plinth area was not relevant; (b) whether **accentuated water shortage** was the cause for increased N by volume in storey III was difficult to ascertain since people opted to replenish than to replace water; lack of association between the volume of water stored and N^+ was consistant with earlier studies (Focks *et al.*, 1981; Subra, 1983); (c) an **ecological 'catastrophe'** such as drought could augment and consequently alter the observed patterns of N,N^+. Present studies followed years of reduced rains in Pune viz., 55.68 cm. in 1982 and 57.57 cm. in 1986. The rainfall normal for the city was 66.1 cm. (Indian Meteorology dept., Pune, Unpubl. data). With 50 lit./day water supply per capita, which is increased to 60 lit./day to meet with increased consumption during hot dry months of April–May, Pune can scarcely be a water scarcity area. (discussions at the Pune Municipal Corporation). Although draught conditions were not prevalent in the city, water shortage was perceptible with N, adequate enough to support stable N^+. Further studies in the flats with more number of storeys and / or studies on buildings located on variable ground relief could provide conclusive evidence.

The relative prevalence indices are inadequate to account for the container productivity of *Ae aegypti* through seasons (Focks and Chadee, 1997). Similar studies specific to the multistoreyed housing communities are wanting. But target N remains unaltered either for source reduction strategy or for prevention in view of virtual absence of any seasonal change in N (Reuben, *et al.*, 1978).

Cost of control

Since the BI in storey III were ~5.00, the costs of larval control will be prohibitive (Bang, Y. H. personal discussions). Since a consistant BI > 10 in storeys I & II is

suggested to be of risk for a disease outbreak, an outlay for preventive and/or control inputs would be fitting. Therefore *Ae aegypti* control costs could be optimised by restricted source reduction in lower storeys alone.

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The Searching Efficiency of *Aphidius colemani* Viereck after Visiting Insecticide Treated Plants at Different Time Intervals

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Abstract: The searching efficiency of *Aphidius colemani* Viereck, an effective endoparasitoid of aphids was studied after visiting insecticide treated plants at different time intervals. It was found that after the elapse of the residual period of Ambush, Diazinon and Nicotine at the half strength, mortality of the adult parasitoid was nil. The percentage of parasitism was least for parasitoids which had visited insecticide treated plants, but differences with the control were not always significant and there was no relationship between parasitism percent and adult emergence.

Keywords: *Aphidius colemani*, searching efficiency, residual effect

INTRODUCTION

An examination of a typical study of the searching behaviour of a parasitoid may provide insight into the importance of efficiency of individual fitness. The continuous use of insecticides for the control of aphids has almost inevitably been followed by pest resurgence, outbreak of secondary pests, and development of insecticide resistance in the target insects (Croft and Brown, 1975). These consequences are mainly due to the disruption of the control by natural enemies, including *Aphidius colemani* Viereck, an important and very effective endoparasite of several economically important aphid pests of small grain crops and vegetables (Elliott *et al.*, 1994). Because insecticides can affect insect behaviour their distribution on plants and the density and distribution of pests within and between plants (Trumble, 1985), there is clearly a potential for an interaction between insecticide application and parasitoid foraging. The present study was conducted to determine whether the searching efficiency of *A. colemani* was affected after a visit to a treated plant once the residual period had elapsed.

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MATERIALS AND METHODS

The experiments were conducted in the Department of Biological Sciences, Wye College, University of London, U. K.

Experimental plants

Cowpea (*Vigna unguiculata* L.) was used as the experimental plant and these were grown in plastic pots (10 cm diam × 7.5 cm deep) in normal potting compost in the glass house.

Aphid cultures

Cultures of *Aphis fabae* Scopoli were maintained on faba bean in the stock room of the Biological Sciences Department, Wye College at a temperature of $18 \pm 2°C$ and light (16 : 8 hour L : D day) using fluorescent lamps.

The parasitoid

Aphidius colemani were supplied by the Biological Crop Protection Ltd. (B.C.P.), Wye and collected at the mummy stage. For each searching behaviour experiment, newly emerged adults were allowed to feed on honeydew for 12 hours prior to use. The honeydew was diluted to 1 : 3 (honeydew : water) for experimental use.

Experimental arena

Petridishes (8.5 cm wide and 1.5 cm deep) were used as experimental arena for each experiment.

Procedure

All searching behaviour experiments were monitored using a computer programme "Micromeasure V3". The whole set up comprising a computer, colour monitor, genlock device and video camera. Genlock enables the external video signal to be mixed with the computer programme, allowing the movement of the experimental animals to be drawn directly onto the video picture, so that various parameters can be measured. The movements of insects were followed using a mouse and the stop watch on the monitor was started at the commencement of each replication.

The insecticides Ambush (Pyrethroid), Diazinon (organophosphorus) and Nicotine (natural insecticide) as commercial formulations were used at half the recommended dose, where the recommended doses for Ambush, Diazinon and Nicotine are 0.5 ml a.i./l, 1 ml a.i./l and 1 ml a.i./l respectively.

Two and half-week old cowpea plants were used as the experimental substrate. After treatment with the particular insecticide, the plants were kept for 20, 15 and 4 days for Ambush, Diazinon and Nicotine, respectively before releasing the female parasitoids onto them. The residual period of Ambush and Diazinon was two weeks and the same for Nicotine was one week (Ivens, 1993). The following four treatments were then used:

T1 - Parasitoids which had not visited the treated plants (these were sprayed with distilled water for 1h).

T2, T3, T4 - Parasitoids which had visited the treated plants for 1h, 4h and 6h, respectively.

After visiting the treated plants for 1h, 4h or 6h periods, the parasitoids were collected and released into a perspex cage, 450 × 450 × 500 mm (length × breadth × width), with netting on one side for ventilation, and containing a plastic pot with 2 cowpea plants, each with 20 adult *Aphis fabae*. The behaviour of the parasitoid was then observed for 2h and the number of strikes was counted. The parasitoids were then removed and the plants with aphids were kept for 14–16 days to observe the percent parasitism and the percent adult emergence from the mummified aphids. The experiment was carried out in the laboratory at temperature $18 \pm 2°C$. Each treatment was replicated ten times. The data were analysed using one-way ANOVAs and t-tests. In addition, arcsin transformation was used to compare means of percent parasitism and percent adult emergence.

RESULTS AND DISCUSSION

Percent mortality

Mortality of adult female *A. colemani* was nil after visiting the leaves treated with Ambush, Diazinon and Nicotine.

Mean number of strikes

Ambush

The mean number of strikes by freshly emerged (control) parasitoids was significantly greater than for those which had visited the treated plants ($p < 0.001$). However, there were no differences between the three insecticide treatments (T2, T3 and T4), although the trend was for fewer strikes the longer the parasitoid was on the lead (Table 1).

Diazinon

The number of strikes was greatest with the control parasitoids but was only significantly greater than those spending 6h on the treated plants ($p < 0.05$). The means for the three insecticide treatments did not differ significantly and trends relating to time spent on the treated leaf were unclear, but parasitoids on the treated leaf for 6h had the least number of strikes (Table 1).

Nicotine

The results were similar to those for Ambush and Diazinon. The number of strikes was greatest for parasitoids which had not visited the treated plants, although this was only statistically greater than those which had visited the treated plants for 4h ($p < 0.05$). There were no clear trends associated with increasing periods on the treated leaf (Table 1).

Besides, the means for T1, T2 and T3 did not differ significantly between the insecticide treatments. However, the number of strikes was significantly fewer for those visiting Diazinon treated plants than those visiting Ambush or Nicotine treated plants for 6h ($p < 0.05$) (Table 1).

Table 1: Comparison of the number of strikes of *A. colemani* on leaves variously treated with 1/2 concentration of insecticide

Time spent on treated leaves	T1 0h Mean±SE	T2 1h Mean±SE	T3 4h Mean±SE	T4 6h Mean±SE
Ambush	41.6 ± 2.01a	34.5 ± 2.16b	30.7 ± 2.20b	29.8 ± 1.97b
Diazinon	36.1 ± 2.66a	30.2 ± 2.69a	30.6 ± 1.34ab	26.4 ± 2.64b
Nicotine	38.1 ± 2.09a	33.8 ± 2.19ab	31.2 ± 1.28b	34.6 ± 1.57ab

Means in each column followed by a different letter are significantly different at $p < 0.05$.

Percent parasitism

Ambush

This was highest (82%) in T1 and was significantly reduced in the 4h and 6h treatments but not after 2h ($p < 0.05$) (Table 2). The trends were declining in relation to increasing time spent on the treated leaves.

Diazinon

The results were similar to those with ambush except that the percent parasitism for the parasitoids from the untreated plants was significantly greater than for the other treatments (T2, T3 and T4) ($p < 0.01$) (Table 2).

Nicotine

In this experiment there was no singificant difference in the present parasitism between any time treatment (Table 2).

The highest and lowest percent parasitism for these three experiments were 82% and 64%, respectively. When comparisons were made among the insecticides, all treatments except T1 means were found significantly different for Diazinon.

Percent adult emergence

The percent adult emergence was not significantly different between any time (Table 2). However, comparison of treatment means between insecticides showed that treatments T1 and T4 were statistically different ($p < 0.05$) (Table 2).

In all the present experiments, mortality was nil. There were, however, significant differences between the mean numbers of strikes, with a slight trend for fewer strikes the longer the time spent on the treated leaves. With regard to percent parasitism, no differences were observed between the Nicotine treatments, but all the means were significantly reduced with Diazinon compared to the control ($p < 0.01$). However, in the experiment using Ambush, the means became significantly reduced with the control only after 4h on the leaf ($p < 0.05$). When only one strike was allowed per aphid, (Vevai, 1942) observed that only 23.2% became parasitized, two strikes gave 45.7% and three strikes gave 80% parasitism. The results of the present study look similar, with the percentage parasitism increasing with increasing numbers of strikes. There was no apparent relationship between percent parasitism and percent adult emergence.

Table 2: Comparison of the percent parasitism and percent adult emergence (in parenthesis) of *A. colemani* on leaves variously treated with 1.2 concentration of insecticide

Time spent on treated leaves	T1 0h	Arcsin transformation in radians Means±SE	T2 1h	Arcsin transformation in radians Means±SE	T3 4h	Arcsin transformation in radians Means±SE	T4 6h	Arcsin transformation in radians Means±SE
Ambush	82.0	0.97 ± 0.04a	79.0	0.91 ± 0.02a	76.0	0.86 ± 0.03a	75.5	0.86 ± 0.03a
	(90.24)	(1.17 ± 0.08ab)	(81.64)	(0.98 ± 0.08a)	(85.5)	(1.10 ± 0.12a)	(78.80)	(0.96 ± 0.08b)
Diazinon	76.5	0.88 ± 0.05a	64.5	0.71 ± 0.05b	64.0	0.70 ± 0.04b	64.5	0.70 ± 0.03b
	(86.92)	(1.06 ± 0.04a)	(76.74)	(0.96 ± 0.09a)	(81.25)	(1.04 ± 0.09a)	(75.78)	(0.87 ± 0.06a)
Nicotine	80.5	0.94 ± 0.04a	75.0	0.86 ± 0.05a	71.0	0.80 ± 0.06ab	71.5	0.82 ± 0.07a
	(94.40)	(1.36 ± 0.09a)	(86.66)	(1.05 ± 0.04a)	(87.32)	(1.16 ± 0.09a)	(88.81)	(1.19 ± 0.09a)

Means in each column followed by a different letter are significantly different at $p < 0.05$

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House Frequenting and Host Seeking Mosquitoes in a Forest Fringed Village of District Dibrugarh, Assam

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Abstract: Year long light trap collections in human dwellings of a forest fringed village yielded 30 species of house frequenting, host seeking mosquitoes in 6 genera. *Anopheles kochi*, *An. dirus*, *An. vagus* and *An. nivipes/philippinensis* amongst anophelines and *Culex pseudovishnui*, *Cx. vishnui*, *Cx. fuscocephala*, *Cx. tritaeniorhynchus* and *Cx. bitaeniorhynchus* amongst culicines were the commonly collected house frequenting mosquitoes whose seasonality and correlation with monthly rainfall has been described.

Keywords: Broken forest, light trap, mosquito abundance, seasonality

INTRODUCTION

Transmission of mosquito-borne diseases are governed by a variety of specific ecological, epidemiological, geographical and social factors. The north-eastern state of Assam is endemic for the mosquito borne diseases with uneven distribution pattern. While malaria is highly endemic in Barak valley and lower Assam areas (Sharma *et al.*, 1996), the Japanese encephalitis is more prevalent in upper Assam particularly in districts situated in north bank of Brahmaputra (Baruah and Mahanta, 1996); filariasis is localized in tea garden population (Dutta *et al.*, 1995) and sporadic cases of dengue virus activity have been recorded from a couple of places in upper Assam (Baruah and Mahanta, 1996). Even within a small geographical area there are wide differences in the prevalence of mosquito-borne diseases which are primarily governed by the local factors. It is, therefore, evident that the epidemiology of any mosquito-borne disease in an area can be better understood if entomological information pertaining to that area is available. Keeping this in view an entomological study was undertaken during 1995–96 in a typical broken forest ecosystem village of district Dibrugarh, Assam to generate information on the abundance and seasonal prevalence of house frequenting, host seeking mosquitoes in human dwellings and results are presented in this manuscript.

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MATERIAL AND METHODS

The investigations were carried out in village Soraipung under primary health centre Tengaghat in the district Dibrugarh from August 1995 to July 1996. Soraipung village (27°3′N and 95°4′E approximately) is an isolated forest fringed village situated in tropical, evergreen rain forest known as 'Soraipung forest range' at an altitude of 152 M between oil towns of Duliajan and Digboi. The population of the village was 401, comprising of marginal farmers and ex-tea garden labourers living in thatched, mud plastered huts. The average yearly rain fall of the study area was about 2800 mm with premonsoon rains beginning in March. June to September was the monsoon period accounting for 70% of the yearly rainfall. November to February was the cool dry period.

The house frequenting, host seeking mosquitoes were collected at monthly frequency from 2 to 3 human-dwellings (1 fixed and 1 or 2 random) with the help of battery operated CDC light traps. The light traps were operated from dusk to dawn in the human-dwellings at the height of 8–10 feet from the ground level. The number of persons sleeping in the catching stations varied from 4–7 in different months. Mosquitoes collected over night in the traps were carried to the laboratory next morning and identified using the standard keys. Prevalence of mosquitoes was expressed in terms of density per trap.

RESULTS AND DISCUSSION

A total of 1037 mosquitoes belonging to 6 genera and 30 species with the density of 41.5 per trap were captured in 25 trap night collections during the study (Table 1). The proportion of anophelines in light trap catches was 43.8% (9 species) with per trap density of 18.2 and that of culicines (21 species) 56.2% with 23.3 mosquitoes per trap. Amongst the 5 genera of culicines, genus *Culex* was represented by 11 species (51.5%) genus *Mansonia* by 4 species (2.1%); genus *Aedes* by 4 species (1.9%), genus *Armigeres* by 1 species (0.5%) and genus *Coquillettidia* again by 1 species (0.04%).

Mosquitoes of genus *Anopheles* and *Culex* together accounted for 95.3% of the total collections. Within genus *Anopheles*, *An. kochi* was the predominant (15.9%) species closely followed by *An. dirus* (14.6%). *An. vagus* (7.3%) and *An. nivipes/philippinensis* (2.9%) were next in abundance. As far as *Culex* genus is concerned *Cx. pseudovishnui* (15.7%), *Cx. vishnui* (9.9%), *Cx. fuscocephala* (9.5%), *Cx. tritaeniorhynchus* (3.8%) and *Cx. bitaeniorhynchus* (3.5%) were five commonly collected house frequenting species in the study area. The remaining 21 species of mosquitoes were collected occasionally in very low numbers. Monthwise densities and seasonal fluctuations of the 9 common house frequenting mosquitoes is given in Table 2.

An. dirus was the only malaria vector present in light trap collections with the mean density of 6.1 per trap. This mosquito showed distinct seasonality. Its population built up, with the onset of pure monsoon rains, started from March onwards to reach the peak in July, remained high till October and thereafter, with the cessation of rains, it came down gradually and became zero between December to February i.e. cold dry months. The correlation between total rainfall of the month and the density of *An. dirus* was not significant ($r = 0.430$, critical value 1 tail $0.05 = \pm 0.499$). Anil Prakash *et al.* (1997) reported a significant correlation of *An. dirus* density with the

Table 1: House frequenting mosquitoes captured in light traps ($n = 25$) in Soraipung village.

Sl no.	Species	No. collected	Abdominal Condition				% to total collect.	Per trap density
			UF	FF	SG	G		
1.	*Aedes aegypti*	1	0	0	1	0	0.1	0.04
2.	*Ae. lineatopenis*	2	2	0	0	0	0.2	0.08
3.	*Ae. nigrostriatus*	6	6	0	0	0	0.6	0.24
4.	*Ae. caecus/vexans*	11	8	2	1	0	1.1	0.44
5.	*Anopheles aconitus*	1	1	0	0	0	0.1	0.04
6.	*An. annularis*	2	2	0	0	0	0.2	0.08
7.	*An. barbirostris*	12	4	8	0	0	1.2	0.5
8.	*An. dirus*	152	49	103	0	0	14.6	6.1
9.	*An. hyrcanus gp*	15	14	1	0	0	1.4	0.6
10.	*An. kochi*	165	95	70	0	0	15.9	6.6
11.	*An. nivipes/philippinensis*	30	21	9	0	0	2.9	1.2
12.	*An. tesselatus*	2	0	2	0	0	0.2	0.08
13.	*An. vagus*	76	40	5	17	14	7.3	3.0
14.	*Armigeres kuchingensis*	5	5	0	0	0	0.5	0.2
15.	*Coquillettidia spp.*	1	1	0	0	1	0.1	0.04
16.	*Culex bailyi*	2	2	0	0	0	0.2	0.08
17.	*Cx. bitaeniorhynchus*	36	28	5	2	1	3.5	1.4
18.	*Cx. fuscanus*	1	1	0	0	0	0.1	0.04
19.	*Cx. fuscocephala*	99	48	50	0	1	9.5	3.96
20.	*Cx. gelidus*	52	17	35	0	0	5.0	2.1
21.	*Cx. mimeticus*	1	0	1	0	0	0.1	0.04
22.	*Cx. pseudovishnui*	163	73	87	2	1	15.7	6.5
23.	*Cx. quinquefasciatus*	8	3	4	0	1	0.8	0.3
24.	*Cx. tritaeniorhynchus*	40	24	15	1	0	3.8	1.6
25.	*Cx. vishnui*	103	48	47	7	1	9.9	4.1
26.	*Cx. whitmorei*	29	23	5	0	1	2.8	1.2
27.	*Mansonia annulifera*	2	1	0	1	0	0.2	0.08
28.	*Ma. dives*	6	4	2	0	0	0.6	0.24
29.	*Ma. indica*	1	0	1	0	0	0.1	0.04
30.	*Ma. uniformis*	13	5	8	0	0	1.3	0.52
Total		1037	525	460	32	20	100.0	41.5

amount of rainfall occurring two weeks prior to the collections and explained the seasonal fluctuation of this species on the basis of phenomenon of 'horizontal pulsation' exhibited by it.

The density of *An. vagus* was significantly correlated with the monthly rainfall ($r = 0.869$, critical value 1 tail $0.05 = \pm 0.499$) resulting in high prevalence of this species in monsoon months and low in cool dry months. Its peak density was attained in the month of August. Similar seasonal population changes were noted in this species in Philippines (Schultz and Hayes, 1993). However, in north western Orissa, India, Chand *et al.* (1993) recorded two peaks of *An. vagus* in the months of March and August.

The seasonal prevalence of *An. kochi* and *An. nivipes/philippinensis* showed similar pattern. July was the month of their peak abundance. Low density of these mosquitoes were seen in post monsoon months which were further reduced to zero in cool, dry months. However, the correlation of monthly rainfall with the density

Table 2: Monthwise densities of common house frequenting mosquitoes in Soraipung village.

Month	Rainfall (mm)	No. Trap collections	Per trap density									
			Common Anophelines (An.)					Common culicines (Cx.)				
			All mosquitoes	dirus	vagus	kochi	nivipes/philip-pinensis	vishnui	pseudovishnui	tritaeni-orhynchus	fuscocephala	bitaenio-rhynchus
1995												
Aug	646.7	2	29.0	3.5	13.5	2.5	1.5	1.5	0.0	1.0	1.0	4.5
Sept	538.7	3	83.6	12.7	5.3	0.3	2.0	9.3	21.7	4.7	0.3	2.7
Oct	68.6	2	22.0	10.0	0.5	0.5	0.5	1.5	0.0	0.5	1.0	0.0
Nov	30.5	3	7.0	2.0	0.3	0.0	0.0	1.0	0.7	0.0	0.3	0.3
Dec	29.3	2	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1996												
Jan	30.4	2	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Feb	44.3	2	11.0	0.0	0.0	0.0	0.0	1.0	4.0	6.0	0.0	0.0
Mar	282.5	2	13.0	1.0	0.0	0.0	0.0	2.0	6.0	2.0	0.0	0.0
Apl	141.1	2	14.0	2.0	1.5	0.5	0.5	3.5	4.5	0.0	1.0	1.0
May	291.9	2	41.0	8.0	8.0	2.0	0.5	3.5	12.0	0.5	0.5	2.5
June	170.0	2	31.5	10.0	2.0	4.0	1.0	2.5	11.0	0.0	0.5	3.0
July	422.4	1	400.0	39.0	8.0	145.0	16.0	41.0	21.0	6.0	89.0	5.0
Total	2696.4	25	41.5	6.1	3.0	6.6	1.2	4.1	6.5	1.6	3.9	1.4

of *An. kochi* ($r = 0.302$) and *An. nivipes/philippinensis* ($r = 0.403$) was poor and insignificant (critical value 1 tail $0.05 = \pm 0.499$).

Culex mosquitoes collected in light traps in human-dwellings did not exhibit any distinct seasonality. However, all common species of *Culex* viz. *Cx. vishnui*, *Cx. pseudovishnui*, *Cx. tritaeniorhynchus*, *Cx. fuscocephala* and *Cx. bitaeniorhynchus* were more prevalent in monsoon months with their peak density occurring in the month of July. Similarly, cool dry months were the period of their least abundance. Correlation of densities of *Cx. pseudovishnui* ($r = 0.545$) and *Cx. bitaeniorhynchus* ($r = 0.813$) with the rainfall was significant whereas, *Cx. vishnui* ($r = 0.407$), *Cx. fuscocephala* ($r = 0.295$) and *Cx. tritaeniorhynchus* ($r = 0.365$) were poorly correlated (Critical value 1 tail $0.5 = \pm 0.499$) with the monthly rainfall. Similar findings of peak population of mosquitoes belonging to *Cx. vishnui* group during monsoon months were also reported from India (Reuben, 1971). Philippines (Schultz and Hayes, 1993) and Indonesia (Olson *et al.*, 1983).

The present study, though preliminary in nature, provides useful information on prevalence of house frequenting mosquitoes in a broken forest environment along with seasonal fluctuations of a few common mosquitoes. The physiography and climate are two well known important factors determining the seasonality of majority of mosquitoes. In addition irrigation has been demonstrated to play an active role in influencing the mosquito prevalence and density (Yadav *et al.*, 1989). The presently studied village and its nearby areas had no facility of irrigation and a few small fish

rearing ponds and unused kuccha wells were the only semi-perinial mosquito breeding habitats available in the village. However, innumerable number of small pits, pools, ditches, puddles and animal foot prints filled with rain water in the adjacent forest area were the chief sources of mosquito breeding. Except the cool dry months i.e. November to February the study area received regular precipitation in all other months and also recorded the high mosquito densities. Therefore, it can be assumed that variation in seasonal abundance of mosquitoes, by and large, depended on rainfall pattern and availability of the breeding habitats. Moreover, monsoon and postmonsoon months provided the most favourable ambient conditions for mosquitoes' survival and multiplication resulting in high densities in these months. Further detailed study on man-mosquito contact with reference to transmission dynamics of various vector borne diseases in broken forest as well as deep forest environment is suggested to understand the disease epidemiology and planning vector control measures.

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Insecticidal Performance of a Neem Product in Control of Two Major Seed Pests of Forestry Tree Species

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Abstract: A neem derivative Fortune Aza 0.15%, has been evaluated for its protective effect against *Bruchidius* sp. and *Caryedon serratus*, forestry seed pests of *Acacia nilotica* and *Tamarindus indica*. Adult emergence, mortality and oviposition of the pests declined due to the treatment. The longevity of adult and pupal periods were drastically reduced in 75–100 ppm as compared to control.

Keywords: Fortune aza, *A. nilotica, T. indica, Bruchidius* sp., *Caryedon serratus*, behavioural responses.

The usefulness of the neem product for pest management and its pest control properties in terms of its effect on feeding behaviour, reproductive efficiency and developmental stages of various groups of insect pests have been proved in numerous field trials over the years (Bernays and Chapman, 1977; Sieber and Rembold, 1983; Balu *et al.*, 1997). Like any other crop, forestry tree seeds too are attacked by a number of storage pests viz. *Bruchidius, Caryedon* and *Tribolium* species. According to Joseph and Oommen (1960), the post harvest loss, estimated to be around 10 percent, and is mainly due to stored pests. Neem has proved to be effective in protecting stored products, particularly forestry tree seeds, whose loss if untreated can be high. Such losses are frequent due to the inability to apply expensive chemical pesticides. Also, use of chemical pesticides in control of insect pests often leads to ecological backlash in the form of toxic residues, pollution and pest resurgence. Moreover, very limited studies have been initiated on biological activities of neem on seed pests of forestry trees. The results presented here offer further evidence of impact of environmentally friendly and economically viable product of a neem based formulation Fortune aza 0.15% against the storage pests *Bruchidius* sp. and *Caryedon serratus* were observed to be a serious pest of pods and seeds of *Acacia nilotica* and *Tamarindus indica*.

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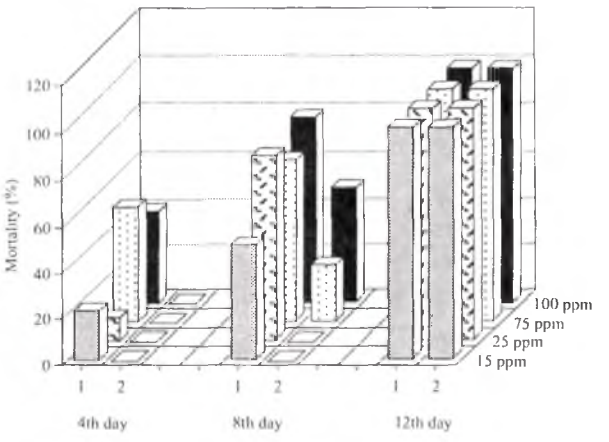

Fig. 1: Effect of Fortune Aza and percent mortality of *Caryedon serratus*. (1) *Acacia nilotica* seed pest (*Caryedon serratus*); (2) *Tamarindus indica* seed pest (*Caryedon serratus*)

MATERIALS AND METHODS

Stock culture of *Bruchidius* sp. and *Caryedon serratus* were maintained in the laboratory on *A. nilotica* and *T. indica* seeds separately and required number of adults beetles (1st day old) were selected for the experimental purpose. Seeds were disinfected in the oven at 40°C for 4hr and kept at the room temperature before use (Santhoy and Rejesus, 1975). Different concentrations of the neem formulations viz., 15, 25, 75 and 100 ppm were prepared with distilled water and used in the test.

Different doses of Fortune aza were sprayed separately on 100 g seeds in glass jars. Control treatment consisted of seeds mixed with water. Required number of adults and pupae were collected from stock culture and exposed to the treated seeds. The number of dead insects and adult emergence from pupae were counted every 24 h. to estimate the adult mortality and pupal emergence. Thereafter, the jars were kept to observe the antiovipositional effect. The number of dead and live insect in each jar was counted at the end of their oviposition to determine the rate of survival. Similarly, damage assessment was carried out on treated and untreated seeds and percentage weight loss in terms of infestation was calculated by the method of FAO (1985). The data obtained from the experiment were subjected to DMRT for statistical significance (Winer, 1971).

RESULTS AND DISCUSSION

Storage pest attacks were the main causal agents in the decline of seed production. In particular, *Bruchidius* sp., *Caryedon serratus* were found to cause serious damage to *A. nilotica* and *T. indica* seeds and the damage was estimated to be 95%. Seeds of *A. nilotica* had a higher rate of infestation than *T. indica* in the laboratory study. Mucunguzi (1995) recorded the high intensity of feeding in small seeds of *A. gerrandii* than the large seeded.

The insecticidal performance of the neem product, Fortune aza has been assessed in terms of antifeedancy, damage assessment, oviposition deterrency, adult emergence (Table 1.) and mortality (Fig. 1). Antifeedancy and insect growth regulation effects

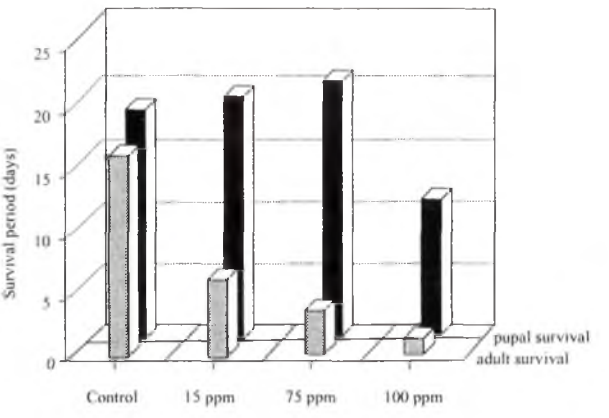

Fig. 2: Effect of FA onthe pural and adult survival periods.

Table 1: Effect of Fortune Aza on per cent Mortality of *Caryedon serratus*

Days after emergence	Mortality			
	15 ppm	75 ppm	100 ppm	control
1–5	–	–	6.7	–
6–10	–	12.9	33.0	–
11–15	9.98	46.9	65.7	–
16–20	59.40	60.0	100.0	100.0
21–25	100.00	61.0	–	–
26–30	–	85.6	–	–
31–35	–	100.0	–	–

were often the most overt effects seen in the treatments of 75 and 100 ppm on *Bruchidius* sp. and *Caryedon serratus*. Antifeedant effects of neem products are well documented (Rembold, 1989) and it has been observed that the pronounced antifeedant activity was shown by relatively higher concentrations of azadirachtin, where as there was little or no antifeedant activity was noted at lower concentrations. *A. nilotica* seeds treated with 75 and 100 ppm exhibited the primary antifeedancy by 96 and 97% respectively than the other concentrations of Fortune aza 15, 25 ppm and control. There is often a secondary antifeedant effect where by the infestation was reduced. Infestation in terms of assessment of damage caused by *Bruchidius* sp. was with 15, 75 and 100 ppm respectively and the damage in *T. indicus* was lesser than the damage in the *A. nilotica*. Seed damage is not always reduced in the lower concentration of neem product viz. 15 and 25 ppm to the same degree as with various synthetic insecticides, however, a clear benefit of neem is that subsequent germination of stored seeds is not impaired by treatment even at 75 and 100 ppm. Seeds treated with higher dosages of Fortune aza, gave 100% protection against attack by bruchid beetles.

The associated behavioural response of *Bruchidius* sp. such as less feeding and reduced or complete inhibition of oviposition, in the various doses of Fortune aza treatments, particularly in 75 and 100 ppm are the important findings from this study.

Besides Fortune aza interrupts insect reproduction in the form of antiovipositional effect is also an important finding of the study. The adverse effect was noted on fertility rate at the concentration of 75 and 100 ppm, as very few females deposited their eggs on treated seeds compared to untreated ones. The fecundity rate was reduced to 42% but not fertility, this might be due to a failure of many oocytes to mature (Tanzubil and McCaffery, 1990). For example, in *Locusta migratoria*, azadirachtin inhibits both oogenesis and ovarian ecdysteroid synthesis thereby preventing oviposition (Rembold and Sieber, 1981). Neem seed extracts and neem oil have been shown to be effective at deterring oviposition of the bruchid beetles, *Callosobruchus maculatus*, *C. analis* and *C. chinensis* (Yadav, 1985).

The higher doses of Fortune aza, 75 and 100 ppm treated *A. nilotica* seeds induced 40–50% adult mortality within 1–3 days and 100% between the period of 8 and 10 days. Similarly, *Caryedon serratus* in *T. indica* showed mortality of 25–50% within 6 days and 100% between 9–12 days (Fig. 1). However, the lower concentrations 15 and 25 ppm were less toxic to bruchids and no mortality was observed on the untreated seeds. The pupal development was not affected significantly in all the concentrations except 100 ppm wherein pupal period recorded 11 days as against 18.5 days in control. However the adult longevity was restricted to 1.3 days in 100 ppm and the survival was reduced in all treatments as compared to control (16.4 days) (Fig. 2). This result corroborates the view of Balu *et al.* (1997) that the formulation, Fortune aza tested was effective at higher concentrations, in particular at 75 and 100 ppm by affecting the survivability of *E. machaeralis*, though some larvae were able to survive in all the treatments and could not reach the adult stages. It is also a well known fact that several plant products not only affect the survival and other developmental stages of insect pests, but also make them available to natural enemies for a longer period and raises the probability of mortality. The foregoing results therefore, clearly indicate the adverse effects of Fortune aza at 75 and 100 ppm on physiology that may reduce the feeding behaviour and survivability.

Neem materials, in spite of possessing broad spectrum activity against stored pests, are generally not hazardous to beneficial organisms, such as predator and parasitoids, and their use could be integrated in stored product pest management.

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Surface Ultrastructure of the Sting in the Rock Honey Bee *Apis dorsata* F. (Hymenoptera : Apidae)

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Abstract: In the rock honey bee, *Apis dorsata* the sting chamber is larger in queen than in the worker. The tergal and sternal hairs are made up of large number of long fibres, slightly twisted together and vary in length in both the female castes. The motor-and sting apparatus also differ in size in the queen and worker bees. Each lancet bears 11 spike-like barbs in the worker bees while they are replaced by 4 blunt barbs in the queen bees. The distal surface of lancets is perforated and bears few peg-like campaniform sensilla in both the female castes. The stylet possesses few peg-like campaniform sensilla. Each sting sheath is covered with blanket of triangular scales bearing group of hairs distally. The scales are perforated in the worker and imperforated in the queen bees.

Keywords: *Apis dorsata*, Female genitalia, Sting sensilla.

INTRODUCTION

In Hymenoptera, the female external genitalia differs widely in structure and function among Chalastogastra and Clestogastra (Snodgrass, 1935; Smith, 1970; Matsuda, 1976). In the social Hymenoptera, however, the female external genitalia consists of motor and sting apparatus distinctly and the structure has been investigated in *Apis mellifera* and some other species of the bees (Snodgrass, 1956; Oeser, 1961; Dade, 1962; Hazeltine, 1967; Hermann, 1971; Hermann and Douglas, 1976; Weiss, 1978; Shing and Erickson, 1982; Jayasvasti, 1989; Mulfinger *et al.*, 1992). Recently conducted surface ultrastructural studies reveal the presence of cuticular processes like barbs, hairs and other sensory receptors on the sting of *Apis mellifera* and some other species of the honey bees (Hermann and Douglas, 1976; Weiss, 1978; Shing and Erickson, 1982).

There is, however, little information available on the structure of sting of rock honey bee, *Apis dorsata* (Jayasvasti, 1989) and the present work was, therefore, undertaken to explore fine cuticular processes associated with the sting of the worker and the queen castes of this species.

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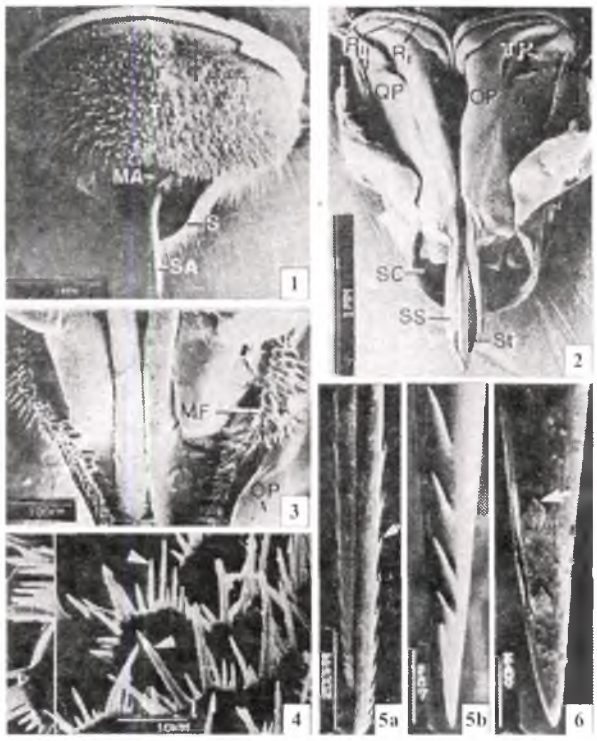

Fig. 1: Sting in a worker bee: T – tergum; S – Sternum; MA – motor apparatus; SA – sting apparatus; Fig. 2: Anatomical organisation of the motor-and sting apparatus in worker bee QP – quadrate, OP – oblong, TP – triangular plates; RI, and R II – ramus I and II. St – stylet; SS – sting sheath; SC – sting chamber; Fig. 3: Membranous fold (MF) and oblong plates (OP) in a queen bee; Fig. 4: Magnified view of hairy lobes showing hexagonal scales with the marginal hairs in a queen bee; Fig. 5: Lancets with spike-like barbs in a worker bee- a) series of barbs in the terminal region (arrows), b) the barbs gradually increasing in size postero- anteriorly; Fig. 6: Lancet with blunt barbs in a queen bee (arrow).

MATERIALS AND METHODS

Large number of adult worker and queen bees were collected from 5–6 daughter swarms of *Apis dorsata* during April-May, 1900–92 from the local (Central India) forest region. The sting was dissected out from the queens and workers and boiled in 10% KOH till the material became transparent. It was then washed, dehydrated, cleared in acetone or clove oil for 3–4 days and studied under stereoscopic binocular microscope. For scanning electron microscopic studies, the cleared organs were mounted on the stubs at different angles with the help of sticking glue "Fevicol". The mounted material was coated with gold in the 'Poloron-gold coating unit' (Dahl, 1972) and scanned under the stereoscan 250 MK III Cambridge scanning electron microscope (SEM) at desirable magnifications at the Regional Sophisticated Instrumentation Centre, Nagpur University, Nagpur (India). The size of the fine structures was measured on the SEM screen and from 25 readings, the mean value and standard error (SE) were calculated.

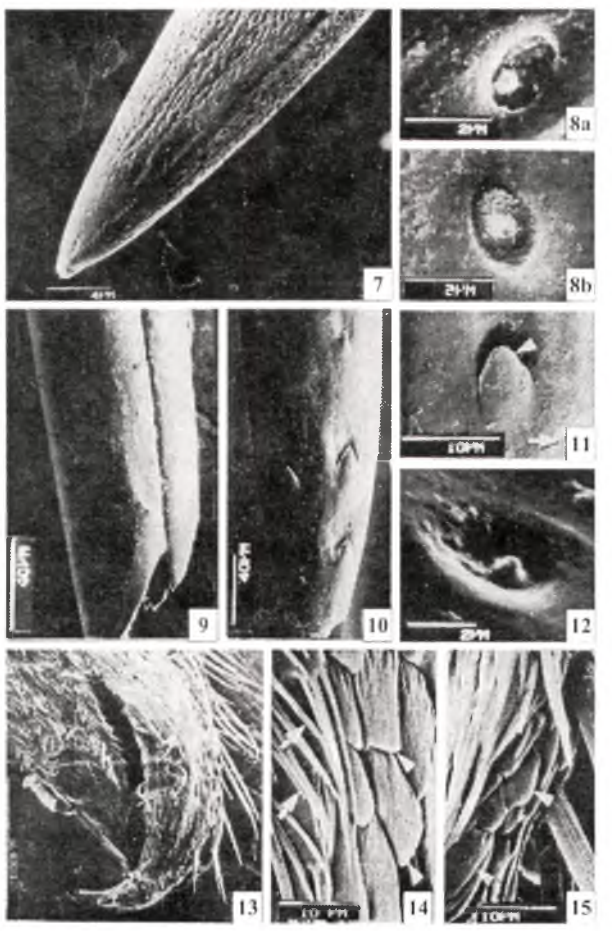

Fig. 7: Distal region of the lancet showing fine perforations all over the surface in worker bee; Fig. 8: Peg-like sensilla on lancet of (a) a worker bee and (b) a queen bee; Fig. 9: Stylet of queen bee showing a bilobed structure; Fig. 10: Three pairs of stylet barbs in a queen bee; Fig. 11: Stylet barb (arrow head) and a peg-like sensillium (arrow) in a worker bee; Fig. 12: Peg-like sensilla on stylet in a worker bee; Fig. 13: Forcep-like appendages of sting sheath in a queen bee; Fig. 14: Perforated scales (arrow heads) bearing the hairs apically (arrows) in a worker bee; Fig. 15: Imperforated scales (arrow heads) on the surface of sting sheath in a queen bee.

RESULTS

Sting chamber

The sting of worker and queen bees is concealed at rest in the conical sting chamber formed by the tergal and sternal plates of segment VII at the terminal end of the abdomen (Fig. 1). The sting chamber is larger in the queen than in the worker bees (Table 1). The tergal margin and inner surface of the sternal wall of the sting chamber are covered with the hairs of variable length (Table 2). The hairs, moreover, emerge

Table 1: Morphometric characteristics of the sting of *Apis dorsata*

Parts of Sting	Worker bee length	Worker bee width	Queen bee Length	Queen bee width
Sting chamber	1330.67 ± 67.14	768.42 ± 38.76	1608.09 ± 82.81	831.53 ± 43.77
Motor apparatus	1816.32 ± 95.43	775.51 ± 27.17	1634.24 ± 89.34	836.57 ± 46.68
Sting apparatus	2857.14 ± 109.48	314.28 ± 21.11	1828.78 ± 92.83	369.64 + 26. 53

Size (μm)
$n = 25$, ± – Standard error (SE) of a mean

Table 2: Caste-specific morphometric variations in the cuticular processes of the sting of *Apis dorsata*

Cuticular Processes		Worker bee	Queen bee
Tergal hairs	L	114.89-425.53	
Sternal hair	L	80.56-319.15	48.32-278.43
Membranous fold hairs	L		
Types MFH1		2.32 ± 0.21	3.89 ± 0.37
MFH2		3.14 ± 0.28	5.98 ± 0.58
MFH3		5.64 ± 0.46	10.18 ± 0.92
MFH4		7.87 ± 0.79	16.47 ± 1.46
MFH5		15.93 ± 1.62	29.94 ± 2.87
Sting Sheath Hairs	L	24.02 ± 2.63	56.77 ± 4.18
Lancet barbs (From the tip)	L		
LB1		21.88 ± 2.2	8.57 ± 0.87
LB2		30.63 ± 2.86	11.14 ± 1.12
LB3		31.25 ± 2.91	13.86 ± 1.37
LB4		39.63 ± 3.86	15.77 ± 1.54
Stylet barbs	L	3.81 ± 8.53	3.92 ± 12.4
Lancet peg-like sensilla	D	2.01 ± 0.14	2.11 ± 0.19
Stylet peg-like sensilla	D	3.93 ± 0.32	3.46 ± 0.29

Size (μm)
Abr : L – length, D – diameter, MFH1–MFH5 – five types of memberanous fold hairs, LB1-LB4 - four types of lancet barbs, $n = 25$, ± – standard error of a mean

out from the apical orifice of the scales spread all over the surface. The tergal and sternal hairs, so far, differ in length in the worker and queen bees. They are similar in diameter ($3.85 \pm 0.32\mu$m, $n = 25$) and are commonly made up of a group of large number of long fibres, slightly twisted together.

Sting

The sting is larger in the worker than in the queen bees (Table 1). It consists of the basal motor-and the distal sting apparatus joined together by the paired transverse rami I and II (Fig. 2).

Motor apparatus

In both, the worker and queen bees, the motor apparatus is composed of paired outer quadrate, inner oblong and apical triangular plates. The entire surface of the quadrate plates is sculptured into the irregular penta-hexa- and septagonal reticulation while that of the oblong plates is differentiated into the overlapping layers of triangular scales. The surface of triangular plates is, however, smooth. The oblong plates possess short socketed hairs (setae) at the anterior margin. They are about 21.7 ± 1.9 and $19.69 \pm 1.8\mu$m in length ($n = 25$) in the worker and queen bees, respectively and are extended over the inner margin of rami II.

The paired membranous folds are lodged in between the oblong plates. They are separated anteriorly and posteriorly while closely apposed at the middle (Fig. 3). The external surface of membranous folds is covered with hexagonal scales having about 5 types of marginal hairs (Fig. 4) and are almost double in length in the queen than in the worker bees (Table 2).

Sting apparatus

In both, the worker and queen bees, the sting apparatus consists of a pair of lancets, a median stylet and a pair of sting sheaths. The lancets are rod-like structures tapering distally and armed with the barbs laterally. Each lancet bears 11 spike-like barbs in the worker bees (Fig. 5 a,b). They are replaced by 4 blunt barbs in the queen bees (Fig. 6). Each blunt barb encloses indentations basally. The indentations are occupied by the dense reticular processes. The surface of lancet in distal region is perforated with the fine pores in both, the worker and queen bees (Fig. 7). A few peg-like sensilla are intermingled with them (Fig. 8 a,b).

The stylet is a bilobed structure, enclosing internally a canal arising from the anterior poison reservoir and opening distally to discharge the poisonous secretion while stinging (Fig. 9). In both the female castes, the stylet is dorso-distally armed with three pairs of blunt barbs (Figs. 10, 11). The stylet barbs are moreover, larger in the queen than in the worker bees (Table 2). Besides the barbs, there are few peg-like sensilla in both the castes (Fig. 12). The proximal region of the lancet and stylet of both the castes is devoid of barbs, peg-like sensilla or pores.

The sting sheaths are long membranous and paired structures, extended over the stylet from the posterior end of the oblong plates. They are broad apically and bifurcated distally into the long forcep-like appendages overlapping terminally with each other (Fig. 13). The plate-like scales are formed all over the surface of the sting sheaths. The scales are perforated due to the presence of fine pores all over the surface in the worker (Fig. 14) while they are imperforated in the queen bees (Fig. 15). The distal margin of the scales is provided with a group of long hairs forming a thick blanket. The hairs are longer in the queen than in the worker bees (Table 2).

DISCUSSION

Although, the sting in female castes of *Apis dorsata* superficially resembles with that of other species of *Apis*, it shows large number of species- and caste specific ultrastructural specialisations, such as i) the reticulated surface of the tergal and sternal hairs by a group of long fibres slightly twisted together, ii) the reticulated surface of the quadrate and scaly surface of the oblong plates, iii) the presence of spike - like barbs on the

lancet of the worker and blunt barbs on the lancet and stylet of the queen and stylet of the worker bees, iv) the presence of peg-like campaniform sensilla on the lancet and stylet of both the worker and queen bees, and v) the presence of a thick hairy blanket over the surface of the sting sheaths in the queen and worker bees.

In *Apis dorsata*, the sting chamber is larger in the queen than that in the worker bees similar to that in *Apis mellifera* (Snodgrass, 1956) which seems to be developed in proportion to the size of the body of both the castes. Snodgrass (1956), moreover, reported the presence of free hairy lobes of the membranous folds connecting the lower margin of the oblong plates of either side in *Apis mellifera*. Most of the earlier studies were made with the help of light microscope which could not reveal the scaly surface of the hairy lobes. The present SEM study reveals distinctly the presence of hexagonal scales all over the surface of hairy lobes of membranous folds extended over the base of sting in *Apis dorsata* and similar fine modifications might be occurring in other species of *Apis*, also. The lancets are armed distally with in all 11 spike-like barbs in the worker of *Apis dorsata* differing from that of *Apis andreniformis, Apis florea, Apis cerana* and *Apis mellifera* workers (Jayasvasti, 1989) and thus signifying the species-specific character. The earlier workers, however, did not report the presence of barbs on the lancets of the queen of *Apis dorsata* while during the present study, 4 blunt barbs are well evident. The stylet of *Apis dorsata* is armed distally with two rows of 3 blunt barbs in both the female castes differing with that of *Apis abndrenioformis, Apis florea* and *Apis cerana* possessing 4–5 spike-like barbs and *Apis mellifera* with 2–4 vestigial barbs (Jayasvasti, 1989). The length of lancet and stylet barbs in both the castes of *Apis dorsata*, however, suggests the species - and caste specific characteristics (Weiss, 1978).

The presence of peg-like sensilla in the distal region of lancets and stylet in both the female castes of *Apis dorsata* has been reported for the first time. They seem to be the campaniform sensilla, alike those described in *Apis mellifera* (Hermann and Douglas, 1976; Shing and Erickson, 1982). They may function as the pressure sensitive receptors (Shing and Erickson, 1982), or may be related to oviposition (Oeser, 1966; Smith, 1972). Similarly, the tip of lancets in both the female castes of *Apis dorsata* is densely perforated similar to that in some members of Hymenoptera and might be related with the mechanism of chemoreception (King and Fordy, 1970). The gross morphology of sting sheaths of the queen and worker bees of *Apis dorsata* resembles with that of other social bees (Snodgrass, 1935; Matsuda, 1976; Hazeltine, 1967; Hermann, 1971; Weiss, 1978; Mulfinger *et al.*, 1992). The present studies reveal the presence of perforated triangular scales in the worker while imperforated ones in the queen bees. Cassier *et al.* (1994) reported the perforated triangular of the sting sheaths in the worker *Apis mellifera* secreting alarm phermone attributed to the defence behaviour and similar caste-specific specialisation of the sting sheaths in *Apis dorsata* workers is also well-evident. In both the castes of *Apis dorsata*, moreover, the scales possess marginal hairs forming a thick blanket over the surface of distal forcep-like appendages similar to that in other species of *Apis*.

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Present Insecticide Susceptibility Status Of *Xenopsylla cheopis* From Beed District, Maharashtra State, India

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Abstract: Insecticide susceptibility studies were carried out on the flea, *Xenopsylla cheopis* in Beed district during the epidemic of fever with lymphadenophathy and post-epidemic seasons. Fleas showed less susceptibility to DDT and Deltamethrin when compared to colony strains and susceptibility to malathion and propoxur was same as to the colony strains of the flea. There was no noticeable difference in the activity of general esterases and acetylcholinesterses in the field and colony strains.

Keywords: *Bioassay, Xenopsylla cheopis, X. astia*

INTRODUCTION

Beed district in Maharashtra state experienced a severe outbreak of fever and lymphadenophathy between August to November 1994 (Dennis, 1994). The outbreak was diagnosed as "plague" by the health authorities. There were reports of unusual flea nuisance and rat fall followed by human cases. The present paper reports the results of the insecticide susceptibility studies carried out on the flea, *Xenopsylla cheopis* collected from the rodents in this district during the epidemic and post-epidemic seasons

Study area

Beed district lies in the central part of Maharashtra state. Administratively, it is comprised of nine talukas (Map 1). This area has relatively low rainfall. The dominant crops are rice, jawar, bajara, sugarcane and cotton. The economy is mainly agrarian with few sugar industries, textile mills and a thermal power station.

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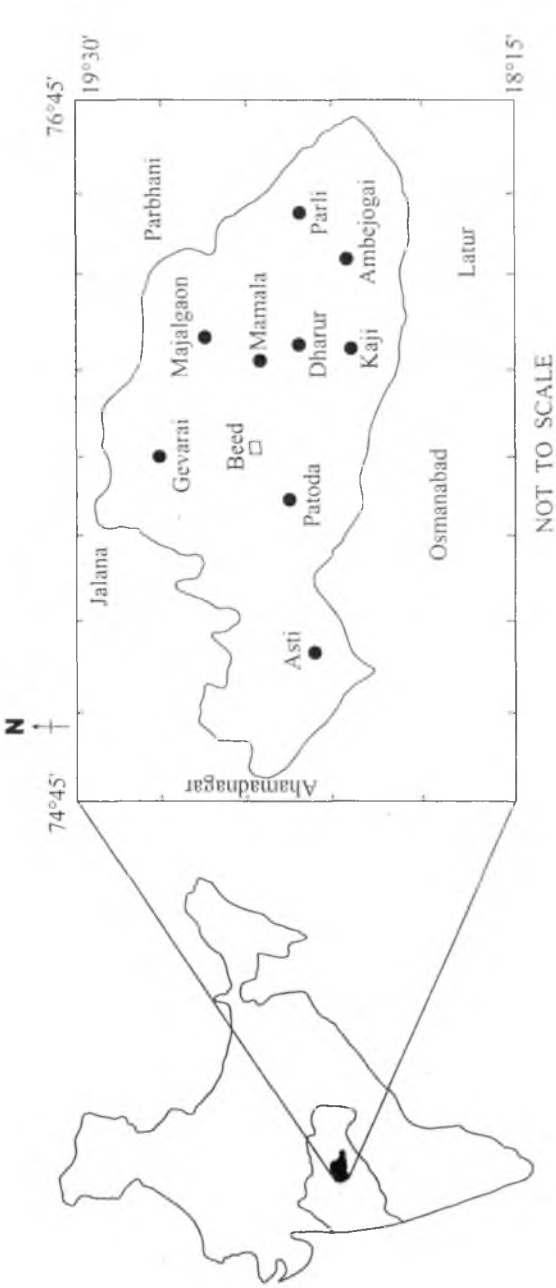

Map. 1 The location of Beed district and position of different talukas.

MATERIAL AND METHODS

Collection of Rodents & fleas

Rodents were collected from all the nine talukas through out the year at the interval of 1–3 months. 'Wonder' and 'Sharman' traps were set overnight in and around human habitations for trapping the rodents alive. Trapped rodents were removed and identified by sex and species and then combed for the collection of fleas in a big plastic container. Fleas were counted and collected in test tubes with the help of an aspirator. *X. cheopis* index was determined as defined by WHO (WHO, 1983).

Insecticide susceptibility test

Susceptibility to DDT, dieldrin, malathion, propoxur and deltamethrin was determined using insecticides impregnated papers. Tests were carried out in the field. DDT 4%, deltamethrin 0.025%, dieldrin 0.4%, malathion 5% papers, were obtained from the WHO. Besides these insecticide impregnated papers were also prepared locally by the method of Busvine & Nash (Busvine and Nash, 1953). Technical grade deltamethrin was procured from Roussel India, propoxur was procured from Bayers India, malathion was procured from Fiscon India Ltd while DDT was procured from Sigma Chemical Co., USA. Test procedure was the same as described by (WHO, 1981). Exposure period for 1% DDT was 24 hrs and for 0.1% propoxur was 5 hrs, however for 4% DDT, 0.4% dieldrin, 5% malathion, 0.025% deltamethrin it was one hr. Test tubes containing fleas were held in wire racks which were kept in the wooden mosquito cages ($30 \times 30 \times 50$ cm.), covered with wet lint cloth. These cages were kept in dark. After the mortality count, fleas were identified and the fleas survived to a particular insecticide were transferred to breeding jars, containing autoclaved rabbit faeces and sand. These fleas were maintained during transit on field caught rodents who were properly combed prior to use as hosts.

Laboratory colony

To determine the base-line data of bioassay and biochemical assays from susceptible insects, fleas were obtained from the colonies of *X. cheopis* & *X. astia* maintained since 1988 at the Institute of Vector Control and Zoonoses, Hosur, Tamil Nadu state. There is no history of exposure of these colonies to any insecticide. Data raised from these colony fleas was used for comparison with field collected fleas.

Enzyme assays

Assays were performed on fleas. Unfed adults were collected from the breeding jars. Fleas were homogenised in 220μl of distilled water per flea with the help of mortar and pestle and centrifuged at 10,000×g for 10 minutes. The protocol followed for esterase (Est A & B) and Acetylcholinesterase (AChE) was same as mentioned in an earlier communication (Mourya et al, 1992). The protein content was estimated in 40μl of supernatent fluid from each individual homogenate by the method described by Lowry (Lowry et al, 1951). A reference standard protein curve was prepared using Bovine Serum Albumin fraction 5.

Table 1: Prevalence of *X. cheopis* in various Taluka of Beed District

Taluka	No. of villages positive for cheopis/surveyed	*X. cheopis* index	Total flea examined	Total *R. rattus* examined
Ambejogai	1/1	3.58	43	12
Asti	1/1	6.48	162	25
Beed	3/5	4.22	249	59
Dharur	2/2	2.22	49	22
Gevrai	1/2	9.31	149	16
Kaij	1/2	3.00	6	3
Majalogaon	2/3	3.53	114	34
Perli	1/1	8.25	165	20
Patoda	3/3	5.21	172	33
Total	15/20	4.97	1109	223

Table 2: Percentage of rodents positive for *X. cheopis*

Rodent species	No. examine	Positive (%)	No. of Flea (*X. cheopis*)
Domestic situations			
Rattus rattus	206	140 (67.96)	1130
Millardia meltada	Nil	Nil	Nil
Indica	Nil	Nil	Nil
Mus musculus	17	1 (5.88)	1
Suncus murinus	24	10 (41.66)	31
Peri-domestic situations			
Rattus rattus	25	5 (20.00)	18
Millardia meltada	36	1 (2.77)	1
Tetera indica	4	Nil	Nil
Mus musculus	4	Nil	Nil
Suncus murinus	58	4 (6.89)	4

RESULTS

Rodents & flea occurrence

A total of 1109 fleas were examined on 223 *Rattus rattus* species. The cheopis flea index ranged between 2.22 (Dharur) to 9.31 (Gevrai) (Table 1). The predominant species in the domestic area were *R. rattus, Mus musculus* and *Suncus murinus*, whereas in the peri-domestic areas *S. murinus* and *Millardia meltada* were found predominant (Table 2) and a few Tetera indica were also collected which showed infestation of *X. astia* along with *X. cheopis*.

Table 3: Percent mortality in *X. cheopis* after exposure to different insecticide impregnated papers

	Percent mortality/(No. tested)					
Insecticide	DDT	Malathion	Deltamethrin	Propoxur	Control	
concentration	1.0%	5.0%	0.025%	0.1%	OP	OC
Exposure Time	24 hrs	1 hr	1 hr	5 hrs	5 hrs	24 hrs
X. cheopis (colony)	88.3 (111)	100.0 (50)	95.7 (70)	100.0 (48)	1.7 (59)	0.0 (23)
X. astia (colony)	90.4 (52)	100.0 (34)	100.0 (34)	100.0 (28)	2.6 (38)	3.9 (26)
	During epidemic					
X. cheopis (Field)	ND	90.0 (30)	84.6 (26)	ND	0.0 (5)	0.0 (5)
	Month after the epidemic					
X. cheopis (Field)	57.7 (52)	100.0 (58)	69.8 (53)	ND	0.0 (11)	0.0 (15)
	Year after the epidemic					
*X. cheopis** (Field)	79.2 (57)	100.0 (47)	78.7 (76)	92.7 (59)	7.1 (56)	ND

* = Corrected mortality
ND = Not done

Susceptibility status to insecticides

Organochlorine compound

During the outbreak period a few fleas were tested with 4% DDT (1 hr exposure) and 0.4% dieldrin (1 hr exposure), which showed 50% ($n = 10$) and 38.7% ($n = 31$) mortality respectively (data not included in the table). A month subsequent to the epidemic, when bioassay were conducted with 1% DDT showed resistance (57.7% mortality) as compared to colony stain, however a year after the epidemic the mortality with 1% DDT was only 79.2% (Table 3).

Organophosphorus and carbonates compounds

X. cheopis showed high susceptibility to malathion and propoxur, only instance when *X. cheopis* showed 90% mortality at the time of epidemic.

Deltamethrin

Percent mortality with deltamethrin was 11.1% to 25.9% less in the field collected strain as compared to colony strain.

Biochemical assay

Data show that there was no noticeable difference in the enzyme activities of colony stains of *X. cheopis* and the field collect strain. Moreover the progeny of survivors of 0.025% Deltamethrin also did not show any rise in the general esterases and acetyl-cholinesterases activity (Table 4).

Table 4: Comparative activity of different enzymes in the adults of *X. cheopis*

Flea species	Enzyme activity/(SD)			
	AChE*	AChE (%) inhibiton*	Est-A**	Est-B**
X. cheopis (colony) ($n = 30$)	7381.48 (772.92)	92.97 (0.63)	22.50 (2.64)	14.83 (1.47)
X. cheopis (Field) ($n = 40$)	7192.67 (1152.4)	97.43 (2.64)	20.31 (3.87)	13.38 (2.37)
X. cheopis (F4) ($n = 40$)	6290.84 (1031.4)	96.45 (2.54)	19.72 (2.47)	13.75 (2.61)

* = Activity/min/mg protein
** = Activity nmol/min/mg protein
(F4) = F4 Progency of the fleas survived to exposure to deltamethrin.

DISCUSSION

Renapurkar in (1990) showed that there is an increase in *X. cheopis* infestation than *X. astia* on the rodents from Maharashtra state and resistance to DDT and malathion (Renapurkar, 1990), however during the present studies it was observed that *X. cheopis* infestation on rodents was almost 99% in all the nine taluka of Beed and only DDT resistance. Results show that there was only one instance during epidemic when the mortality with malathion was 90%, which could be attributed to the degradation of insecticide, since during the epidemic, 5% malathion impregnated paper used for the test were outdated. However, results obtained subsequently with locally prepared 5% malathion impregnated papers showed 100% mortality in the field and colony strains.

While in the case of deltamethrin, during epidemic time old lot of deltamethrin impregnated papers were used which showed less mortality than colony strain, however afterwards, freshly prepared 0.025% deltamethrin papers and freshly procured deltamethrin papers from WHO also showed less mortality in the field strains as compared to colony strain. WHO specification on the discriminating doses for deltamethrin and malathion is not available, hence when compared the results with the colony strain it appears that there is indication of low level of resistance to DDT & deltamethrin and fleas are susceptible to malathion and propoxur. This could be due to use of DDT and deltamethrin in epidemic and post-epidemic periods. On the other hand the colony fleas which do not have any history of exposure to any insecticide also did not show 100% mortality to DDT and deltamethrin, moreover the biochemical analysis of the flea strains also did not show any difference, Hence at this juncture it is difficult to say exactly whether low mortality observed in the field strains of the fleas with DDT & deltamethrin is due to insecticide resistance or any other physiological factor(s).

There is very high cheopis index in many of the areas in Beed district. Our studies show that still fleas are susceptible to many of the insecticides but studies are needed to monitor the vector resistance to different insecticides used for public health and biochemical studies should be done in depth to find out the basis of insecticide resistance in the fleas.

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Potential of using trona (urao) of the control of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) infesting cowpea seeds in storage in Nigeria

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Abstract: The potential of using solutions and powder of trona (urao, sodium sesquicarbonate) to protect edible and proteinaceous seeds of cowpea from infestation and damage by the notorious seed beetle, *Callosobruchus maculatus* (F.) was investigated. Trona solution applied to eggs on seeds and larvae or pupae in seeds generally reduced adult emergence from treated beetle 2-day old eggs and 3-day old larvae but not older larvae and pupae. It inhibited oviposition by the beetle, and the number of adults emerging from eggs laid on treated seeds was significantly reduced. Trona powder at 2.0 g/20 g of cowpea was as effective as pirimiphos methyl at 0.5 g/20 g of seed in reducing egg laying, adult emergence and thereby the control of *C. maculatus* infestation and damage to the seeds. Neither Trona solution nor powder adversely affected cowpea seed germinability.

Keywords: Trona, cowpea, *Callosobruchus maculatus*, control, Nigeria

INTRODUCTION

The cowpea seed beetle, *Callosobruchus maculatus* (F.) is the most serious pest of cowpea, *Vigna unguiculata* (L.) Walp. in West Africa, where the crop is mostly cultivated and serve as the major source of protein in human diet (Murdock *et al.*, 1997). The edible proteinaceous seeds are frequently ravaged by the beetle in storage rendering them unfit for human consumption. Many synthetic insecticides have been reported as effective in the control of *C. maculatus* damage to cowpea seeds. However, several factors including prohibitive costs of these chemicals and inconsistent supplies, safety of workers and consumers, and possibility of the beetle developing resistance, appear to make the use of alternative control methods more attractive. Murdock *et al.* (1997) described several technologies including co-storage of cowpea with abiotic materials of *C. maculatus* control which may eliminate the use of insecticides and could have economic and health benefits for the applicators, consumers and the environment. Trona (Urao) is a naturally occurring sesquicarbonate (Na_2CO_3. $NaHCO_3$. $2H_2O$) in several parts of the world (Harley, 1981), and is used chiefly as food additive

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among households in many African countries. It is known locally as Kaun in Nigeria and Kanwe in Ghana. As a food additive, it may be added to okra (*Abelmoschus esculentus* Moench.) soups to increase the mucilaginous "drawing" quality of the okra fruits, or it may be added to cowpea seeds to reduce the cooking time (Ankrah and Dovlo, 1978). In northern parts of Nigeria, local cattle herdsmen add a quantity of trona to the drinking water of their livestock (Iwunze, 1988). Thus, the mineral is relatively non-toxic to humans and livestock. Emebiri and Nwufo (1988,1990) have shown that trona may have considerable potential for reducing depredation by pests to stored gains. The effect of trona on infestation and damage to stored cowpea seeds by *C. maculatus* is reported in this paper.

MATERIALS AND METHODS

Beetle culture

The *C. maculatus* used was derived from a colony originating from infested cowpea seeds collected from a local market in Akure, Nigeria. The colony has been maintained in Kilner jars in a cooled incubator at $30 \pm 1°C$ and $50 \pm 5\%$ relative humidity for more than 30 generations, using life Brown cowpea (a known susceptible variety) as substrate.

Trona solutions

Lumps of trona were obtained from a local market in Akure, Nigeria and were crushed manually using mortar and pestle. The following concentrations: 0.25%, 0.5%, 0.75%, 1.0%, 5% and 10% were prepared using water as solvent. The concentrations were arbitrarily selected.

Effect of different trona solutions on developmental stages of beetle

The development stages of beetle tested were eggs on seeds, and larvae and pupae in seeds. The various stages were treated at the following ages: eggs (2 days old), young larvae (3 days after egg hatch), mature larvae (about 12 days old as larvae) and pupae (2 days old as pupae). To obtain eggs of *C. maculatus* on seeds, twenty pairs of freshly emerged adults were placed in a Kilner jar containing about 400 Ife brown cowpea seeds. Five kilner jars were so set up so that there will be sufficient seeds bearing the different developmental stages to be used. The adults were allowed 24 h to lay eggs on the seeds. Seeds with egg loads of two to four eggs on the seeds. Seeds with egg loads of two to four eggs per seed were selected. Fifty eggs on seeds or 50 larvae or pupae in seeds were placed in separate plastic petri plates (8.0 cm diameter). Seeds containing larvae were set up by selecting hatched eggs determining by the change of their colour to milky white because of the deposit of freaks by the feeding larvae (Giga and Smith, 1987). The position of the pupae were easily detected from the transparency of testa due to feeding habit of last larval instar and their number was easily assessed. Treatment of 50 eggs on seeds or 50 larvae or pupae in seeds with each concentration of trona solution constituted a replicate. The developmental stages were treated by dipping the seed in the solution. For the control, 50 eggs on seeds or 50 larvae or pupae in seeds were dipped in tap water. Treated seeds were subsequently air dried in their respective petri plates. Four each concentration of salt solution and the control there were four replications. After air drying the petri plates were placed

in the cooled incubator and observed for adult emergence. the total number of adults emerging from each replicate was recorded.

Effect of trona solution on beetle oviposition and subsequent adult emergence

Choice and no-choice tests were used to determine oviposition preference of *C. maculatus* for untreated seeds and those treated with trona solution. Ife Brown cowpea was used for the choice experiment because the brown seed coat permits easy visibility of bruchids eggs. Seeds of similar size (visually assessed) were used. Three hundred of the seeds were dipped in the 10.% trona solution and another three hundred in tap water. Both lots were air dried and equilibrated to ambient laboratory conditions. The experiment involved giving a freshly emerged mated female of *C. maculatus* the choice of laying either on trona treated or water treated seeds which were mixed together in the same Petri plate. Fifteen seeds of each kind were provided as oviposition substrate in the Petri plate. The trona treated or water treated seeds were marked at the hilum area for easy identification in different replicates. After 24 h, the number of eggs laid on the seeds of each kind were counted. There were fifteen replications of this experiment.

In the no-choice experiment, 100 trona treated and 100 water treated seeds (as described above) were placed in seperate Petri plates. The seeds were then infested with a freshly emerged copulating pair of *C. maculatus*. After the death of the female, the number of eggs laid on the seeds were counted. The experiment was replicated 10 times. The total number of adults emerging from each replicate was also recorded 35 days after infestation.

Efficacy of trona powder in reducing beetle infestation and damage to cowpea

Trona lumps were crushed into powder and 0.5, 1.0 and 2.0 g portions were weighed. Each portion was mixed with 20.0 g Red Drum cowpea (another susceptible variety) in test tubes. These doses/rates fall within the range which have been tested for many powdery substances tested for bruchid control in legumes. A freshly emerged couple of *C. maculatus* was introduced into each tune. Any couple that did not initiate egg laying after 24 h was replaced. Three were five replications per treatment. A standard treatment involving using 0.5 g pirimiphos-methyl (Actellic dust) per 20.0 g of seed was also set up in five replications. An untreated replicated control was similarly set up. Number of holed seeds, seeds bearing eggs and adults emerged were recorded per replicate 38 days after infestation.

Effect of treatment with trona solution with powder on germination of cowpea seeds

The trona solutions tested were of concentration: 1.0%, 5.0% and 10.0%. Lots of freshly harvested Ife Brown cowpea seeds were separately treated with the different rona solutions, and powder at the rate of 2.0 g of seed. The seeds were kept in the cooled incubator. Germination trial was carried out after three months. Ten treated seeds were germinated in a Petri plate containing moistened filter paper. Germination count was taken after eight days. There was a control treatment involving dipping freshly harvested seeds in water. All treatments including the control were replicated five times.

Table 1: Mean percentage adult emergence (± S.E) of *C. maculatus* from seeds treated with different concentrations of solution of trona

% of trona in solution	Mean Percentage adults emergence from treated: 2 day old eggs	3 day old eggs	12 day old larvae	2 day old pupae
Control	54.6 ± 5.53	76.6 ± 3.26	63.2 ± 1.48	58.8 ± 3.81
0.25	43.6 ± 3.48	49.2 ± 0.93	61.2 ± 1.88	57.6 ± 2.48
0.50	53.6 ± 5.92	42.8 ± 4.64	60.8 ± 1.69	58.8 ± 1.24
0.75	40.0 ± 3.70	42.8 ± 4.64	60.4 ± 2.54	60.4 ± 1.26
1.0	34.4 ± 2.19	39.2 ± 3.30	58.8 ± 2.21	61.2 ± 1.24
5.0	42.4 ± 3.02	36.0 ± 2.24	52.0 ± 3.74	58.0 ± 1.63
10.0	32.8 ± 3.68	21.2 ± 2.42	54.8 ± 4.23	53.6 ± 5.58
LSD (0.05)			6.23	

Table 2: Oviposition and adult emergence of *C. maculatus* involving cowpea seeds treated with water (Control) and 1% solution of trona in the no-choice experiment

Treatment	Mean No. of eggs laid on seeds in 24 hr (± S.E)	Mean No. of eggs laid on seeds after death of female(± S.E)	Mean percentage adult emergence from eggs laid (± S.E)
Control	27.7	58.6	66.3
1% Trona solution	13.3	23.3	44.6
P*	< 0.05	< 0.05	< 0.05

* By one-way ANOVA

RESULTS

Effect of different trona solution on developmental stages of beetle

There were significant ($P < 0.05$) differences in the percentage of adults that emerged from eggs, larvae and pupae of *C. maculatus* treated with trona solution of different concentration and water (control) (Table 1). All concentrations of trona solution significantly reduced adult emergence from treated 2-day old eggs and 3-day old in comparison with the control. The 10% trona solution significantly reduced adult emergence from treated eggs and young larvae more than other concentrations. Adult emergence percentage from treated 12 day old larvae and 2 day old pupae was not significantly ($P < 0.05$) different from the control except with 12 day larvae treated with 5% and 10% solutions of trona where fewer adults emerged.

Effect of trona solution on beetle oviposition and subsequent adult emergence

In the choice experiment a significantly ($P < 0.05$) greater number of eggs were laid on control seeds that on seeds treated with trona solution in 24 h (Table 2). In the no-choice experiment total eggs laid on control was similarly significantly greater than the number laid on seeds treated with trona solution. A significantly larger number of adults emerged from eggs laid on control seeds than from eggs laid on seeds treated with trona solution.

Table 3: Infection and damage by *C. maculatus* to 20 g of Red Drum cowpea seeds mixed with different amounts of trona powder in comparison with pirimiphos-methyl (Actellic dust)

Amount of Material per 20 g of seed	Mean % of holed seeds ± S.E	Mean % of seeds bearing eggs ± S.E	Mean number of adults emerged ± S.E
0.5 g trona	31.2 ± 4.59	66.4 ± 3.31	44.4 ± 2.60
1.0 g trona	18.4 ± 1.33	58.4 ± 3.87	29.4 ± 3.72
2.0 g trona	11.2 ± 1.62	13.6 ± 1.43	12.8 ± 2.08
0.5 Actellic dust	10.8 ± 1.50	11.6 ± 1.33	10.4 ± 0.74
Control	62.8 ± 2.87	76.6 ± 2.89	54.8 ± 4.42
LSD (0.05)	5.59	5.91	6.26

Table 4: Germination of cowpea seeds after three months of treatment with trona powder and solutions

Treatment	Mean% germination
1% trona solution	80.0 ± 2.24
5% trona solution	70.0 ± 5.48
10% trona solution	68.0 ± 3.74
Trona powder	78.0 ± 3.74
Control	78.0 ± 3.74

ANOVA indicated that $p > 0.05$

Efficacy of trona powder in reducing beetle infestation and damage to cowpea

Summary of data on infestation and damage by *C. maculatus* to 20 g of Red Drum cowpea seeds mixed with different amounts of trona powder in comparison with pirimiphos-methyl (Actellic dust) is presented in Table 3. Mean percentage of holed seeds, mean percentage of seeds bearing eggs and mean number of adults that emerged were significantly ($P < 0.05$) higher in the control than in the seeds treated with trona powder and Actellic dust. The value for the damage parameters were not significantly ($P < 0.05$) different in treatment involving use of 2.0 g trona powder and 0.5 g pirimiphos-methyl. Damage to protected cowpea seeds significantly increased with decreased amount of trona powder used.

Effect of treatment with trona solution and powder on germination of cowpea seeds

Treatment of cowpea seeds with either solution or powder of trona did not affect the germinating capacity of the seeds (Table 4). Mean percentage germination ranged from 68 to 80.

DISCUSSION

This study has shown that trona may be useful for storing cowpea seeds to prevent it from devastation by *C. maculatus*. Both the solution in water (containing 10% trona) and the ground powder (at 2.0 g per 20 g of seed) were found to be most effective in reducing infestation and damage to cowpea seeds. For the solution, the mechanism

of action appear to be contact toxicity to eggs and young larvae as well as ability to inhibit oviposition by the beetle. Older larvae and pupae of *C. maculatus* were observed to be resistant to the effect of trona solution. For the powder, the design of the experiment does not permit elucidation of the mechanism of action with certainty. Apparently, only a few seeds could be infested at the effective level of 2.0 g trona powder per 20 g of cowpea seeds. While the powder at this rate appeared to act as a physical barrier, preventing the beetle from reaching many seeds, it is possible for trona to impact the beetle in other ways. For instance, it may have acted as desiccant for the eggs as has been suggested for wood ash (Akpeatok, 1974). The beetle may have deliberately avoided seeds bearing trona dusts. Results of the choice experiment indicated a definition avoidance behaviour in *C. maculatus* towards seeds treated with trona solution. Antennal palpation of the seeds during the patrol phase of the oviposition behaviour or the beetle would reveal the presence of extraneous materials. Emebiri and Nwufo (1990) investigated the insecticidal potential and biological activities of trona dust against *Sitophilus zeamais* (Motsch). They observed that trona dusted grains were avoided and that the dust was highly lethal to adults. Fecundity of *S. zeamais* was also reduced in grains treated with trona dust relative to the untreated control. Trona dust did not appear to exercise may significant toxicity to *C. maculatus* adults in this study. It was casually observed that all couples lived for more than four days during which they could have concluded their normal reproductive activities (Wassermann, 1985)

The use of powdered trona may be preferred to the solution because of the additional energy and time required to dry seeds treated with the solution. However, the abundance of sunshine in the tropics means that drying cowpea seeds immersed in trona solution is feasible. In fact, it is common practice among households and traders to periodically expose infested cowpeas to sunshine in tropical Africa, presumably to reduce pest load. The presence of one or two holes and a few adults in treated cowpea seeds could make it more difficult to convince potential users that trona is effective, but it is common knowledge and experience that occurrence of low infestation does not seriously alter the acceptability of cowpeas for food or seed among tropical small holders and households (Van Huis, 1991; Wolfson *et al.*, 1991). The observation that trona did not affect the viability of treated cowpea seeds is a d vantageous to its use for reducing *C. maculatus* damage in storage.

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Evaluation of the Efficacy of 'Stored Grain Insect Trap'

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Abstract: Efficacy of the 'Stored Grain Insect Trap' (Mohan *et al.*, 1992), has been tested on heavily infested paddy and wheat grains collected from various parts of Palakkad district, Kerala, India. The study revealed encouraging results in that it helped to trap 100% of the larvae and adults of 4 species of pests, and larvae of 2 species which are highly injurious. Collection of larval forms with this trap is reported for the first time.

Keywords: 'Stored Grain Insect Trap', Infestation, Larval pests.

INTRODUCTION

Insect pests have been a threat to food and seed ever since man has started growing crops. Almost all stored food stuffs and seed are liable to insect injury (Saxena, 1995). The protection of stored grains from insect pests is of considerable importance owing to their severe infestation and damage in a short period (Swamiappan *et al.*, 1976c). Use of chemicals as a means to control infestation is hazardous as far as human health is concerned (Omkar, 1994), and hence emphasis is being given to efforts to develop new techniques, using non-toxic substances. Alexander *et al.* (1944), Krishnakumari and Majumdar (1964), Majumdar and Venugopal (1964), Swamiappan *et al.*,(1976a, b and c) and Venugopal (1975) have studied the efficiency of certain inert materials in controlling infestation in stored commodities. Majumdar *et al.*, (1959,1962) have tested materials such as activated clay, charcoal and silica gel for their protectant properties against storage pests.

Stored grain insect trap or 'Probe Trap' is a mechanical device developed by Mohan *et al.* (1992) which helps in the artificial control of stored grain pests. It is designed to make use of the natural behaviour of insects to enter holes. The trap is reported to be successful in trapping *Sitophilus oryzae, Tribolium castaneum, Rhizopertha dominica* and *Laemophlocus minutus* (Mohan *et al.*, 1992). Present work has been executed with a view to evaluate the efficacy of the trap in question against stored grain pests.

MATERIALS AND METHODS

The probe trap was procured from TNAU, Coimbatore, Tamil Nadu, India. Infested paddy and wheat were collected from different parts of Palakkad district, Kerala, India.

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A 50/50 mixture of samples was prepared and kept in the laboratory for one week for acclimatisation. Six samples, each weighing 1 Kg, were taken in large glass jars and marked A, B, C, D, E and F. The traps were slowly pushed into the samples. Care was taken not to kill or injure any pest and to place the trap at the centre. Mouth of the jar was closed using linen with loose mesh in order to facilitate aeration. It also prevented the pests from escaping. The settings were kept undisturbed. The traps were taken out once in two days, pests were sorted and counted. Observations were continued till all the pests were trapped.

OBSERVATIONS

Four species of adult pests belonging to the order Coleoptera viz., (1) the lesser grain borer *R. dominica* (Bostrychidae), (2) the rice weevil *S. oryzae* (Curculionidae), (3) the saw-toothed grain beetle *Oryzaephilus surinamensis* (Silvanidae) and (4) the red flour beetle *T. castaneum* (Tenebrionidae), have been identified from the collection using the trap. Among these, *T. castaneum* was found in the highest density (49.46%), followed by *R. dominica* (28.26%). *O. surinamensis* and *S. oryzae* were present only in much less density (Table 1). In addition to the adult pests, some larval pests were also trapped during the present study. They were identified as the larvae of the Angoumois grain moth *Sitotroga cerealella* (Gelechiidae) and the meal moth (rice moth) *Corcyra cephalonica* (Galleriidae), both belonging to the order Lepidoptera. The larvae constituted about 6.25% of the total collection (Table 1). All the pests were trapped within a period of 10 days. More than 50% of the pests were found to be trapped within two days (Table 1 and 2). 76% of *R. dominica* and 69.5% of *T. castaneum* were eliminated during the first observation itself. It was interesting to note that the larvae of *C. cephalonica* comprised about 60% of the total larval forms and those of *S. cerealella* about 25%. The rest were those of the coleopteran adult pests present in the sample.

DISCUSSION

Fumigation and spraying various chemicals are the usual practices adopted to prevent infestation by pests (Metcalf and Flint, 1990; Omkar, 1994). They also provide the names of certain household insecticides also. However, such methods are hazardous as far as human health is concerned. They are also expensive, for domestic purposes. Omkar (1994) has given a detailed account of the impact of pesticides on human health. The inert dusts tested by Alexander *et al.* (1944), Krishnakumari and Majumdar (1964), Majumdar *et al.* (1959), Majumdar and Venugopal (1964), Swamiappan *et al.* (1976a, b and c) and Venugopal (1975) could produce high mortality of pests. But it was found that they tend to lose their efficiency in the course of time (Swamiappan *et al.*, 1976c). This necessitates the addition of fresh dust after some time which is neither economical nor practical. Cleaning of food grains at the time of consumption is also another problem. The probe trap developed by Mohan *et al.* (1992) is a cheap, convenient, efficient and safe method for checking infestation of small scale storage of grains and seeds. According to him it takes about 14 days to make a good collection. During the present study, however, 100% elimination of the pests was effected within a period of 10 days, when provided one trap per 1kg of sample. Probably the rate of trapping may vary with the ratio between the number of traps and the quantity

Table 1: Number of insects trapped in five different observations

Sl. No.	Names of pest	Samples	Observations					Sample Total	Percentage Occurrence
			I	II	III	IV	V		
1	*Tribolium castaneum*	A	62	4	2	3	2	73	
		B	30	9	3	4	1	47	
		C	64	30	1	2	1	98	
		D	41	8	2	4	2	57	
		E	18	15	1	3	2	39	
		F	38	8	0	4	0	50	
	Total		253	74	9	20	8	364	49.46%
2	*Sitophilus oryzae*	A	12	1	0	2	1	16	
		B	8	2	2	0	0	12	
		C	5	6	1	0	1	13	
		D	3	1	4	1	0	09	
		E	2	1	0	0	0	03	
		F	0	0	1	0	0	01	
	Total		30	11	8	3	2	54	7.34%
3	*Rhizopertha dominica*	A	3	8	2	4	2	19	
		B	21	1	3	0	1	26	
		C	68	2	2	3	1	76	
		E	25	2	2	3	0	18	
		F	33	0	1	2	1	37	
	Total		158	19	12	14	5	208	28.26%
4	*Oryzaephilus surinamensis*	A	4	4	0	2	0	10	
		B	3	0	1	2	0	06	
		C	7	4	1	2	1	15	
		D	7	1	0	1	1	10	
		E	3	2	0	2	0	07	
		F	8	6	1	1	0	16	
	Total		32	17	3	10	2	64	8.69%
5	Larvae	A	5	1	1	2	0	09	
		B	2	1	0	0	0	03	
		C	2	5	0	0	1	08	
		E	4	2	2	1	0	09	
		F	5	2	0	1	0	08	
	Total		20	15	5	5	1	46	6.25%
	Grand total							736	

of sample. Attack by different types of pests also vary with varying environmental factors (Pradhan, 1991; Saxena, 1995). *L. minutus*, collected by Mohan *et al.* (1992) using this trap were not present in samples collected from Palakkad.

Feeding is the sole important function of larval forms and growth is very rapid (Lefroy, 1990) and so their role in the desruction of stored grains cannot be overlooked. It is calculated that lepidopterous and coleopteran larvae consume up to 20 times their own dry weight of food (Lefroy, 1990). The larvae of *S. cerealella* and *C. cephalonica* are

Table 2: Percentage of each pest trapped in each observation

Name of the Pest	Percentage of trapping in each observation				
	I	II	III	IV	V
Tribolium castaneum	69.5	20.3	2.5	5.5	2.2
Sitophilus oryzae	55.6	20.4	14.8	5.6	3.7
Rhizopertha dominica	76.0	09.1	5.8	6.7	2.4
Oryzaephilus surinamensis	50.0	26.6	4.7	15.6	3.1
Larvae	43.5	32.6	10.9	10.9	2.2

known to cause severe damage to the stored grains and seeds (Comstock, 1984; Metcalf and Flint, 1962; Nayar *et al.*, 1976). Among the members of Lepidoptera, it is exclusively the larvae that are injurious while among coleopterans they are equally destructive as the adults (Lefroy, 1990; Metcalf and Flint, 1990; Nayar *et al.*, 1976). Pradhan (1991) showed that in *Schistocerca gregaria* and *Trogoderma granarium* the resistance to pesticides is higher among the early stages of life cycle, especially in early larval stages. Above facts points to the need to develop stored grain larval pest control measures, other than chemical, owing to the apparent economical implications. However, this strategic assessment of larval control has not been made by the inventor of the trap, and hence some attention has been paid along this line. An interesting observation made during the present investigation was that larval stages of pests could also be trapped using the probe trap. Since Mohan *et al.* (1992) has not mentioned anything about the trapping of larval forms this seems to be the first report.

The probe trap (stored grain insect trap) has thus proven to be a very successful tool for controlling insect pests, both adults and larvae, in small scale stores of grains and seeds. The contraption may further be modified, after further studies, into an eco-friendly means for the control of insect pest infestation in larger granaries and godowns also.

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Brief Communication

Incidence of *Poekilocerus pictus* (Pyrgomorphidae: Orthoptera) on Some New Hosts in Arid Western Rajasthan

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Abstract: Several new hosts of *Poekilocerus pictus* are reported from the western Rajasthan. Of the most preferred hosts, *Plumeria alba, Tabernaemontana* (Apocynaceae) and *Chrysanthemum maximum* (Compositae) were for feeding and *Moringa oleifera* (Moringaceae) was for adult congregation.

Keywords: *Poekilocerus pictus*, hosts.

Poekilocerus pictus is a common and well-known grasshopper but of little economic significance as a pest. It usually breeds and feeds only on *Calotropis* spp., However, it sometimes increases unusually to cause severe damage to many fruit, vegetable and field crops, and garden ornamentals (Anonymous, 1982). Yousuf and Gaur (1993) reported this grasshopper as feeding on *Acacia senegal, Prosopis juliflora* and *Tecomella undulata*. This report is based on occurrence and feeding of *P. pictus* on some garden plants despite abundant availability of the natural hosts (*Calotropis gigantea* and *C. procera*) in the vicinity.

Since 1995, the nymphs of the grasshopper have been observed to feed upon garden plants at Nandan Van, in the outskirts of Jodhpur (73° 0′E, 26° 20′N). The locality is a relatively new settlement with freely growing shrubs of *Calotropis* and *P. juliflora* in and around newly constructed but uninhabited buildings. The occupied buildings have newly developed or developing gardens close to these wild shrubs within a distance of 2–5 metres. Preferred plants observed were *Plumeria alba* L., *Tabernaemontana divaricata* (L.) R. Br. (Apocynaceae), *Canna orientalis* Rosc. (Cannanaceae), *Chrysanthemum maximum* Ram., *Zinnia elegans* Jacq. (Compositae), *Moringa oleifera* Lamk. (Moringaceae), *Jasminum sambac* (L.) Ait (Oleaceae), *Rosa chinensis* Jacq. (Rosaceae), *Aegle marmelos* (L.) Corr. (Rutaceae) and *Cestrum nocturnum* L. (Solanaceae). Amongst the less preferred hosts were *Polyalthia pendula* (Annonaceae), *Taernae montana divaricata* (L.) R. Br., *Wrightia tinctoria* R. Br. (Apocynaceae), *Leptadenia pyrotechnica* (Forsk.) (Asclepediaceae), *Helianthus annuus* L. (Compositae), *Jasminum arborescens* Roxb. (Oleaceae), *Murraya koenigi* (L.) Spreng. (Rutaceae) and *Cestrum diurnum* L. (Solanaceae).

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The host cross over of the grasshopper from *Calotropis* spp. to the garden plants occurred maximum during April to July when the neo-alates developed and started mating. Mobility of the alates was pronounced. Nymphs, particularly III stage frequently chose to nibble upon the alternate hosts. The average number of grasshoppers per plant was maximum on *Plumeria alba* (3.60) during summers (April–July) and on *Chrysanthemum maximum* (3.75) during winters (October–March). Maximum foliar damage (30–70% of leaf area) was also in *P. alba* and *C. maximum*.

On *Polyalthia pendula* only the nymphs of I to III instars were observed to feed: adults rarely gave but only small bites to the leaves, implying that it was not a preferred host for the insect. Obviously the plants were taken up as hosts in early stages when the nymphs have little inclination to move or migrate to the preferred host *Calotropis*. After III instar, however, the nymphs which start movements, prefer to shift to the preferred host. During May–June, *Moringa oleifera* trees attracted a large number of grasshoppers, especially neo-alates and mature adults, including copulating pairs which prefer to settle on the terminal shoots. The number of insects lodged on a single tree was 47 on 19.5.96. On this tree, the adults were observed to feed upon aerial parts-leaves, flowers, rind of the pods and even bark of young shoots.

It appeared that plants in the family Apocynaceae were generally preferred and many plants in the family Solanaceae also served as food for *P. pictus*, *Moringa oleifera* appeared to strongly attract only the adults for congregation. Although adults were found settled on plants like *Delonix regia*, *Acacia senegal*, *Prosopis juliflora* and *Tecomella undulata*, no feeding was observed.

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Brief Communication

Evaluation of Natural Plant Product as an Insecticide Against Top Borer *Scirpophaga excerptalis* Wlk. In Sugarcane

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Abstract: Field experiment to evaluate the natural plant product as an insecticide against top borer was conducted at G. S. Sugarcane Breeding and Research Institute Seorahi, Padrauna. Extract of all the natural plant product reduced the incidence of top borer. The least incidence (6.35 and 6.17%) was observed with *Eucalyptus rostrata* (3% solution) compared to the check (16.88% and 15.77%) during 1989–90 and 1990–91 respectively. It was followed by *Azadirachta indica* and *Eucalyptus rostrata* (2% solution) during two years.

Natural plant product have attracted considerable interest as potential insect pest management in agriculture. The compound obtained from natural plant product affect insect behaviour viz. repellant chemicals perceived by chemosensory receptors have an impact on insect's nervous system resulting in higher vapour tension which affect the orientational response of the insect towards he host antifeedants (Doharey and Singh, 1989) and oviposition deterrent (Singh and Srivastava, 1983). They also affect the metamorphosis (Sharma et al, 1980) and reproductive fitness of the insects. The isolation of active ingredients from the natural plant product is costly and tedius and the application of pesticides resulting into environmental pollution which affects the human being. Therefore, the crude extracts obtained from various naturally occuring plants were evaluated against *S. excerptalis*, a major sugarcane insect-pest in Eastern U.P..

The flower and fruits of *Nerium indicum*, green leaves of *Eucalyptus rostrata*, *Azadirachta indica* and *Calotropis procera* were collected. One kilogram of each sample were dried in shade and powdered. The powdered sample were dissolved in 500 ml. acetone, stired by electric shaker for 8 hours. The solutions were filtered by whatmans filter paper No. 1. The excess of solvent were evaporated by rotatory evaporator. The crude product obtained were formulated on W/v basis to get desired concentration by adding water.

The experiment was laid out in Randomized block design with three replication during 1989–90 and 1990–91 using three concentration (1,2 and 3%) of plant extracts. The extracts were applied twice against each brood of *S. excerptalis* during egg laying

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Table 1: Evaluation of natural plant product as an insecticide against top borer

Sl. No.	Treatment		% Incidence of top borer			% Incidence at harvest	% Incidence of top borer			% Incidence at harvest	Average inci. 1989-90	Average inci. 1990-91
			Ist br	IInd br	IIIrd br		Ist br	2nd br	3rd br			
1	Spraying of Extract of Kaner fruits against brood (each)	1%	3.42	5.95	8.61	26.36	4.11	6.76	10.87	12.94	11.08	8.67
2	-do -	2%	3.83	4.38	6.82	22.70	4.55	5.39	8.82	10.89	12.57	7.41
3	-do-	3%	3.39	4.33	6.59	27.31	4.17	5.34	8.49	10.56	10.40	7.14
4	Spraying of extract of kaner flower	1%	4.99	6.05	10.60	27.11	5.46	6.80	12.58	14.65	12.18	9.87
5	-do-	2%	5.25	5.71	9.33	29.85	5.26	6.68	12.32	14.39	12.53	9.66
6	-do-	3%	4.02	5.23	8.83	24.00	5.04	6.00	10.74	12.81	10.52	8.64
7	Spraying of extract of eucalyptus leaves	1%	–	–	–	–	4.39	4.97	9.26	11.33	–	7.48
8	-do-	2%	3.41	3.98	6.36	28.23	4.37	5.20	8.26	10.33	10.49	7.04
9	-do-	3%	2.07	3.37	5.76	14.20	3.11	4.12	7.71	9.77	6.35	6.17
10	Spraying of extract of neem leaves	1%	–	–	–	–	4.87	5.16	9.34	11.40	–	7.69
11	-do-	2%	4.10	4.03	6.38	27.97	4.41	4.90	8.37	10.43	10.62	7.02
12	-do-	3%	4.85	4.57	8.00	26.71	4.36	5.15	9.88	11.95	11.03	7.83
13	Spraying of extract of madar leaves	1%	–	–	–	–	3.99	7.04	11.48	13.55	–	9.01
14	-do-	2%	3.52	5.97	8.43	28.76	3.75	6.84	10.44	12.51	11.67	8.38
15	-do-	3%	3.23	5.45	7.78	23.90	3.55	6.39	9.75	11.81	10.09	7.87
16	Control (spraying of detergent only)		6.28	7.96	11.94	31.69	7.97	8.84	14.06	16.13	14.46	11.75
17	Control (spraying of water only)		8.47	9.51	16.10	33.44	10.72	12.58	18.86	20.93	16.88	15.77

N: S. CV = 24.57 = 13.18 = 48.35 = 41.44 = 19.31 = 16.25
SE = 2.15 3.50 1.70 1.86 2.05 2.07
CD = 4.64 7.57 3.44 3.76 4.36 4.39

period. The percent incidence of top borer was recorded in each brood to evaluate the insecticidal efficacy.

Extract of all the natural plant product reduced the incidence of top borer (Table 1), which varied with the extract. The least incidence (6.35 and 6.17%) was observed with *Eucalyptus rostrata* (3% solution) compared to the check (16.88% and 15.77%) during 1989–90 and 1990–91, respectively. It was followed by *Azadirachta indica* and *Eucalyptus rostrata* (2% solution) during two consecutive year.

Foliar application of different plant extracts during egglaying period of each brood (2nd and 3rd) resulted in reduction of top borer infestation which might be due to ovipositional deterrent (Singh and Srivastava, 1983) and egg hatchability of insects. Krishna and Pathak (1987) observed that odour of non-host plants effected breeding potential decline the egglaying and hatchability of larvae. Similarly Varun et al (1994) observed that odour of spices crops reduced the incidence of shoot borer.

Hence, the extract of natural plant may be exploited as an insecticide in Integrated Insect-pest Management to control the top borer (*S. excerptalis*) in sugarcane.

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Brief Communication

A New Report of *Achroia grisella* Fb. (Lepidoptera: Galleriidae) as a Seed Pest of Bamboo Reed (*Ochlandra ebracteata* Raizada & Chatterjee)

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Abstract: *Achroia grisella* Fb. is reported for the first time as a seed pest of the bamboo reed *Ochlandra ebracteata* Raizada & Chatterjee in natural forest stands in Kerala, India. The caterpillars of this insect tunnelled irregular cavities in the fleshy pericarp of fresh seeds lying on the forest floor. Feeding by this insect often injured the embryo inside and upto 10.08% of seeds were found to be thus damaged.

Keywords: *Achroia grisella, Ochlandra ebracteata*, seed pest, bamboo reed, Kerala, India.

Achroia grisella Fb. belonging to the lepidopteran family Galleriidae is principally a pest of beehives, affecting beeswax and is widely distributed in the Palaearctic, Oriental and Australian Regions (Hampson, 1896; Lefroy, 1909). The adult moths measure about 3–3, 5 cm across the wings and are uniformly brown in colour, with a yellow head. The larvae which are brownish in colour measure about 4 cm in length and are active, feeding under a web made of wax and excreta. At Achenkoil in Kerala, the larvae of this moth were found to attack the fruits of the bamboo reed *Ochlandra ebracteata* Raizada & Chatterji lying on the forest floor. The fruit of *O. ebracteata* is a *bacca* characterised by the presence of a fleshy pericarp. There is no endosperm and the space between the pericarp and the embryo is filled with 'aleurone' tissues. The seed longevity is only for about four months. Although the fruits initially showed very high moisture content, on the forest floor the moisture content was lost very rapidly and the fruits soon became wrinkled and hard.

Only fleshy fruits with high moisture content were preferred by *A. grisella*. The larvae tunnelled in to the fleshy pericarp making an irregular cavity in the seed which was stuffed with frass and excreta. The larval tunnelling often injured the embryo inside and the affected seeds failed to germinate. Field samples showed that 10.08% of the seeds were thus damaged.

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The fruits of *O. ebracteata* were also eaten by wild pigs. The fruits were dried and powdered for use as cattle feed by the local people. Appasamy (1993) has shown that the fruit contains mostly starch, protein, sugar and phenols.

O. ebracteata is an erect shrubby reed bamboo with tufted clumps of about 60 to 70 culms. In Kerala, culms of this species is in great demand for various commercial uses, (for making baskets, mats etc.) and the seeds are procurred for raising seedlings for planting. In bamboos, as the flowering occurs gregariously at long intervals and the culms die after the seed fall, the subsequent regeneration is dependant on the seeds formed. Seed predation by various organisms is therefore very important in the silviculture of bamboos. At Achencoil, gregarious flowering of this species has been recorded in 1961–1963. Next flowering recorded was in 1992, after a period of 30 years (Seethalakshmi, 1993).

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Brief Communication

Biochemical Studies on Digestive Amylase in New Bivoltine Races of Silkworm, *Bombyx mori* L. Comparative Analysis of Yield Traits

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Abstract: Amylase is a key enzyme involved in digestion and carbohydrates metabolism in silkworm and its activity is related to productivity traits. The calorimetric estimation of digestive amylase activity has been taken up in new bivoltine races of *Bombyx mori* viz. NP2, SP2 and KSO1 to adjudicate their superiority if any, over NB4D2 and KA races. NP2, SP2 and KSO1 exhibited activity of 246.2, 231.9 and 223.6 mg of maltadextrin/30 min./20μl of digestive juice where as amylase activity in control races viz. NB4D2 and KA was 189.4 and 197.6 mg of maltadextrin/30 .min./20μl of digestive juice respectively.

Keywords: Silkworm, *Bombyx mori*, Amylase, Yield traits.

Quantitative traits of commercial importance in the silkworm *Bombyx mori* L. have exhibited different heritability estimates due to environmental effects in addition to genetic factors (Chatterjee et al, 1992). Amylase is one of the key enzymes involved in digestion and carbohydrate metabolism in insects (Horie and Watanabe, 1980). The association between digestive amylase activity and some commercial characters of importance is well established by the earlier workers (Ashwath et al, 1992; Chatterjee et al, 1992). Thus an attempt has been made to evaluate in detail the genetic variability of amylase activity of new bivoltine races evolved at KSSRDI, Bangalore and its relevance to the yield traits in tropics. The newly evolved bivoltine races, viz., NP_2, SP_2 and KSO_1 and existing popular bivoltine races viz., NB_4D_2 and KA (control) were used for the present study. The silkworm rearing was done as detailed by Krishnaswami (1986), using (6–8) layings, brushed *en-masse* and in third instar, they were replicated with 300 larvae in each rearing bed. Yield parameter viz., single cocoon weight, single shell weight and effective rate of rearing (ERR) were analysed in each replicate. All samples were collected 3–4 hours after 15th feeding in the fifth instar if not otherwise stated. Following the procedure standardised by Chatterjee et al (1988), the enzyme activity was assayed for each replication of the samples in three separate

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Table 1: Digestive amylase activity vis-a-vis quantitative traits in Control and New Breeds

Race	Activity of Digestive amylase #	ERR (%)	Single cocoon weight (g)	Shell ratio (%)	r
NP2	231.7	81.63	1.58	18.91	+0.935 *
SP2	231.7	82.3	1.56	18.45	+0.810 *
KSO1	223.6	78.90	1.57	18.63	+0.160 *
NB4D2 ($)	189.4	73.50	1.70	19.56	-0.999 *
KA ($)	197.6	70.80	1.64	19.28	-0.998 *

$ Control races
significant at 1% C.D
μg of maltadextrin released/30 min/20μl of digestive juice

test tubes and for each test tube 2 ml of starch solution (2 mg/ml pH - 9.2) was used as substrate for 20μl of digestive juice. The incubation was done at 37°C for 30 min. and the reaction was stopped by adding 2 ml of 3,5 dinitrosalicyclic acid (E. Merck).

The colour was developed by keeping the mixture in boiling water bath for 5 min. and was cooled down. The absorbance was noted at 525 nm after cooling and the concentrate was established on the basis of standard curve of maltose. The amylase activity is expressed as mg of maltadextrin released/30 min/20μl of sample. Correlation analysis was done to establish the relationship between the digestive amylase activity and ERR (Table 1).

Results of primary analysis of the amylase activity in the digestive juice is shown in Table 1. The evaluation of yield parameters showed that the effective rate of rearing in the new races namely NP_2, SP_2 and KSO_1 was 86.52%, 62.30% and 78.9% respectively which is significantly higher than the existing traditional bivoltine races namely NB_4D_2 and KA (73.5% and 70.8%) respectively). However the values of single cocoon weight and shell ratio of the new races are lower than that of NB_4D_2 and KA 1. On the other hand the activity of digestive amylase is significantly higher in NP_2, SP_2 and KSO_1 exhibiting 246.2, 231.9 and 223.6μg of maltadextrin released/30 min/20 ml of digestive juice respectively, where as in NB_4D_2 and KA it is 189.4 and 197.6μg/30 min/20μl of digestive juice respectively (Table 1).

The association between digestive amylase activity and certain quantitative traits in *B. mori* is well established (Hirata, 1974). Based on the preliminary observations of the correlations between digestive amylase activity and yield traits, Chatterjee et al (1989) suggested the importance of carbohydrate metabolism towards yield realisation in silkworm and this contention is substantiated by the results of the present study.

Medium activity of digestive amylase in the new races coupled with better survivability observed in the present study corroborates the work done by Hirata (1974). Digestive amylase with its highly significant positive correlation with survival (measured as ERR %) seems tailor made for the situation which could be used as a marker in breeding for higher survival. In the present study, medium activity of digestive amylase in the new races coupled with higher ERR compared to control races is an indication that the new races possess higher survivability and moderate productivity which are the need of the hour. Hence, positive correlation between survival potential

and amylase activity in new races, therefore, is a significant finding deserving attention of the silkworm breeders in India.

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